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Embryological Studies of Certain Teleost Fishes With Special Reference Tothe Possible Significance of Melanophores in Piscine Taxonomy.

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EMBRYOLOGICAL STUDIES OF CERTAIN TELEOST FISHES WITH SPECIAL REFERENCE TO THE POSSIBLE SIGNIFICANCE OF MELANOPHORES IN PISCINE TAXONOMY

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ABSTRACT

The present work is an embryological study of certain egg-laying freshwater teleost fishes, with special reference to the significance of developing melanophores in piscine taxonomy. Nine species of tropical fishes, representing four families and three orders, all well known to aquarists, were reared in the laboratory. These include, according to Schultz (1955), Hyphessobrycon serpae (Characidae); Brachydanio rerio, Brachydanio albolineatus, Brachydanio nigrofasciatus and Tanichthys albonubes (Cyprinidae); Oryzias latipes (Cyprinodontidae); Betta splendens, Trichogaster trichopterus and Colisa lalia (Anabantidae). Observation of developmental stages, drawings and charts are included.

The eggs and early stages of embryogenesis are presented. For the study of melanophores four arbitrary stages were selected - initial, primary, transitional, and final stages of embryonic pigmentation. These stages are not static but merge one into the other in varying degree of rapidity. For regional comparison certain key areas on the embryonic and larval body were selected. Generalizations in each case rest on four batches of at least ten eggs each. Basing stages on a time factor is not reliable, as wide variations occur due to such environmental conditions as temperature, oxygen content of water, and fungus growth on eggs.

In one family, Anabantidae, a greater similarity exists between

eggs of two genera than between eggs of each of these and eggs of the remaining genus. But since egg characters may well be considered adaptive, it would not seem necessarily implied that these similarities support interpretation of relationship.

It is observed that melanophore formation is similar in the species of a given genus. The genera of a family present a different situation. In the cyprinids the two genera, though identical in the initial site of pigmentation (eye), differ from one another in other stages. This initial site (eye) of pigmentation is found in Oryzias latipes (Cyprinodontidae); this suggests a probable relationship between the families Cyprinidae and Cyprinodontidae. In other stages of pigmentation the members of the two families differ.

The three genera of the family Anabantidae on the other hand are unique in having the yolk as the initial site and also in the possession at the initial stage of pigmentation of a comparatively large number of melanophores, suggesting a family character. Similarities in the succeeding stages are greater between Trichogaster trichopterus and Colisa lalia than between either one of them and Betta splendens.

Hyphessobrycon serpae of the family Characidae does not resemble any other species at any stage of pigmentation. The majority of the recognized authorities of piscine taxonomy keep families Characidae and Cyprinidae in a single order. The family

Characidae, is placed however, under a separate order from that of Cyprinidae by Jordan (1923); and the present data at least suggest the correctness of this interpretation.

The overall consideration of the stages of pigment formation in these fishes indicates taxonomic relationship, the indication being strongest in the final stage of pigmentation. In this stage related species resemble each other but may be separated finally by melanophore counts on key areas, by general distribution and by pattern formation.

INTRODUCTION

Fish taxonomists generally agree that the adult coloration, particularly the characteristic markings such as blotches, stripes, bars and spots, is constant within a species and is determined by the heredity of the animal. Exceptions to this are due to seasonal changes, to environmental variations, and to sexual dimorphism. Cases have been reported in which the characteristic markings of the adult vary greatly within a species, as examplified by a species of Trunkfish (Ostracion) from the Barrier Reef of Australia, and Vica (Hypoplectrus) of the West Indies; but it may beconcluded generally, with some exceptions, that the characteristic markings of the adult fishes are useful for species recognition.

Lagler (1952) states that within the species the black or brown melanophores have patterns of distribution on the body and the yolk which are distinctive of the developmental stages. He further states that the location of melanophores in key areas is also characteristic in certain species and is, therefore, useful for their identification. Such studies would be of obvious economic significance, especially in the field, where identification of the species is basic to a sound conservation program.

The present study is an attempt to pursue the matter of embryonic pigment patterns with the objective of determining their constancy and their individuality; and of ascertaining whether these patterns indicate genetic relationship.

Hodges and Behre (1953) made an extensive study of the melanophore development in the embryos of the anabantid fish, Trichogaster trichopterus. They observed that the melanophores first appear on the yolk sac and then on the body shortly afterwards. As the development proceeds they found an increase in number, and described slow amoeboid movement on the yolk sac and on the body. Prior to hatching they also recognized the arrangement of the melanophores into characteristic groups on the body and definite bands in the tail region. They further noticed that the melanophores are paler at first appearance and become darker in later stages of development.

The idea that the melanophore embryology probably would make a major contribution to the identification of the developing eggs and larbae of closely related species is mentioned by Mansueti and Mansueti (1955). These workers also think that the above study will greatly enhance the better management and utilization of certain fisheries. Norman (1948) states that the parr marks[1] are highly characteristic of the larval salmon living in the fresh water. There is, therefore, an indication that the embryonic and larval melanophores and their arrangement into patterns are basic characteristics, hence might be expected to prove of taxonomic value at least as far as the species are concerned.

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[1] By parr marks are understood cross bars characteristic of young salmon.

Regarding the genetics of melanophores, Gordon (1931) and Tavolga (1949) recognize in the Mexican platyfish, Platypoecilus, that the pigmentation of the ocular region precedes that of the brain region. Tavolga (1949) has found that the initial pigmentation of the hybrid between Platypoecilus maculatus and Xiphophorus helleri occurs either at the same stage as in platyfishes in general or earlier.

Melanophores in amphibians, birds and mammals have been so extensively studied that their neural crest origin and subsequent pattern formation are well established. On the other hand, the melanophores of fishes have been much less studied, with the result that satisfactory and critical evidence has been lacking so far to demonstrate their origin and subsequent pattern formation. Most of the studies that deal with the pigmentation of fishes are on color changes and adaptation, on regeneration of color pattern in adult fishes, and on the genetics of pigmentation.

On the whole explanations of the origin of chromatophores in fishes fall into three categories. There are those who believe that the chromatophores originate from the neural crest. Of these Borcea (1909) has been credited as the first person to have observed this. He recognizes these cells between the surface ectoderm and the neural tube in Belone embryos. He also observes that in the case of the embryos of Uranoscopus, Fierasfer, and Labrax these cells are visibly pigmented before migration from the neural crest region to

various parts of the body and of the yolk. Newth (1951), who has made an extensive study on the development of the lamprey embryos, has found that here the pigment cells definitely originate from the neural crest. He suggests very strongly that the development of the lamprey embryos represents the basic pattern for the development of both Agnatha and Gnathostoma; thus fishes might be expected to show the same pigment cell origin. Orton (1953), from her observation on the embryos of forty species of California marine fishes, strongly favors the neural crest origin of the chromatophores in fishes. She finds that their origin and subsequent pattern formation can be traced in vivo in the case of some small and transparent eggs, thus conforming substantially to the claim made by Borcea. Orton further stresses the importance of chromatophore embryology and pattern history as aids to taxonomists. The present author has pursued this point as his main objective. The theory of neural crest origin of the pigment cells in the teleost fishes is supported by the experiments of Lopashov (1944). These experiments include (1) the removal of the presumptive areas of the brain and (2) the transplantation of these brain pieces under the epithelium of the yolk sac. In the first type of experiment he observes either a total absence of or a reduction in the number of the pigment cells in the operated areas; in the second type of experiment pigment formation typical of the brain region results in all those areas to which the brain tissue is transplanted.

Regarding this source of the origin of the melanophores, Stockard (1915) and Spaeth (1916) are strongly of the opinion that at least the dermal melanophores are derived from the mesenchyme cells that wander about in amoeboid manner in the early embryonic stages. Spaeth interprets the melanophores of fishes, reptiles and birds as functionally modified smooth muscle cells, opposing the general opinion that they are specialized connective tissue cells.

There are still those who believe the source of melanophores to be otherwise than previously stated. The experimental work of Goodrich (1950) seems to indicate that the pigment cells, particularly those of the yolk, are derived from the germ ring, therefore precede the development of the definitive crest.

In their analysis of the development of color pattern in the anal fin of the adult Brachydanio rerio, Goodrich, Marzullo and Bronson (1954) have found that the black stripes of the treated areas could be re-formed by melanophores which move in amoeboid manner from the adjoining areas not separated by the yellow stripe. They also observe that the prospective yellow stripe is re-formed by the degeneration of the melanophores in this region while the xanthophores are making their appearance. They, therefore, advance a hypothesis that the xanthophores are effective in delineating the color pattern and that the process is made possible by some kind of chemical substance secreted by the xanthophores at the time of their differentiation from the pro-pigment cells.

Oppenheimer (1950), who worked with germ ring grafts and isolates of some teleost fishes, has found that these tissues give rise to either red blood corpuscles or melanophores or both. She was thus led to the idea that the function of melanophore formation, under certain experimental conditions, is taken over by cells normally not involved in such activity, thus accounting for the contradictory experimental results of Goodrich (1950) and Lopashov (1944). The apparent contradiction can perhaps be reconciled by the concept that the neural crests themselves as well as the pro-pigment cells may be definitely determined in the germ ring.

GENERAL EMBRYOLOGY

For convenience in following the descriptive material a brief summary of the embryology of these fishes is given below.

THE EGG. The species studied like all modern bony fishes have a highly telolecithal egg with the active cytoplasm restricted to one side of the egg after fertilization. This area, which is the animal pole, is commonly known as the germinal disc or blastodisc. The opposite end of the egg forms the vegetal pole. The egg is protected by a kind of membrane which is derived from the ovary or the oviduct. This membrane is closely applied to the egg before fertilization, but it becomes lifted and loses its former proximity to the egg forming as a result a perivitelline space. The egg then becomes freely suspended in the fluid of the perivitelline space.

CLEAVAGE AND BLASTULATION. Cleavage, as would be expected of the highly telolecithal egg, is meroblastic and discoidal. The early cleavage planes are vertical and result in the formation of few blastomeres on the germinal disc. As cleavage continues many smaller-sized cells are formed, and horizontal divisions also set in, forming thus a few layers of cells. At this time a cavity appears between the raised blastodisc and the underlying yolk, the segmentation cavity, which is comparable to the blastocoel of other types of egg. The marginal cells of the blastodisc, which are continuous with the underlying

yolk, later become what is conventionally known as the germ ring. The blastodisc, which is raised above the yolk, forms a cap with a convex surface. With the further increase in size of the segmentation cavity blastulation is complete. A blastula of this type, forming a cap or disc, is called a discoblastula.

GASTRULATION. Gastrulation begins when the marginal cells of the blastodisc thicken to form a well marked germ ring. The segmentation cavity grows wider and the blastodisc becomes reduced in its thickness as the germ ring grows down slowly over the yolk as if to engulf the latter. Soon the embryonic shield makes its appearance by a heavy concentration of cells on one border of the germ ring. The early embryonic shield is broader than long, and it has its base coincident with the margin of the germ ring. This particular region of the germ ring forms the future posterior border of the embryo; it is considered homologous with the dorsal lip of the blastopore of other types of gastrulae. In this way the future dorsal, anterior and posterior border of the embryo are early determined.

NEURULATION. As the germ ring grows further down over the yolk, the embryonic shield elongates anteroposteriorly and narrows from side to side. The neural keel that forms in the center of the embryonic shield differentiates into the neural tube which is soon divisible into the primitive brain and spinal cord, the former being broad and the latter narrow and more uniform. The posterior end of the

embryo at this stage forms a flat tail-bud which is still coincident with the margin of the germinal ring. Somites then begin to form.

EARLY EMBRYO. As the germ ring advanced, making the exposed area of the yolk smaller, the embryo elongates and is now raised slightly above the yolk. The head shows the primary regions of the brain by the characteristic shape of the regions and the constrictions present between them. The eyes and auditory vesicles make their appearance one after the other, the former on the sides of the fore-brain and the latter on those of the hind-brain. The number of somites increases to about twenty, this number varying with the species.

LATE EMBRYO. The germ ring has now passed well beyond the equator of the egg, and the exposed yolk, now often called the yolk plug, is on the point of disappearing. The embryo is definitely fishlike, and its main parts such as the head, trunk and tail are distinguishable. The embryo has become greatly elongate, and is raised above the yolk, partially freeing the head and tail for movement. The lens of the eye is properly fitted in position and the auditory vesicles are each supplied with two otoliths. Pectoral buds make their appearance a little behind the ears. The tail which is now free lies close to the head and flagellates periodically, causing the embryo to swing from side to side. The heart beats regularly and is well formed. Circulation is already effected and blood cells are reddish.

The visible yolk becomes smaller and is oval in outline from the side view. At the posterior part, it has narrowed into a tubelike structure which is cylindrical and is in the process of being incorporated into the primitive intestine.

PRE-HATCHING. The yolk has become greatly reduced and the embryo is larger, its length generally exceeding the greatest circumference of the yolk sac. The eye shows iridescence and the body becomes darker with melanophores. The pectoral fins are well developed and are highly vibratile. The embryo prior to hatching is equipped with organs sufficiently developed for the free life. The tail at this stage vibrates very vigorously with the result that the egg-membrane breaks open at one side to make way for it. By repeated movements of the tail which is now free the head is pushed out of the egg-membrane; thus hatching is accomplished.

MATERIALS AND PROCEDURE

For the purpose of the study ten species of egg-laying freshwater teleost fishes, fairly well known to the aquarists as being hardy, easily available, and prolific in reproduction, were chosen. One of the species, Danio malabaricus (Jerdon), failed to spawn even though the animals were kept under presumably ideal conditions for nearly two years. Fresh stocks were procured from different sources but no eggs were obtained. With the exception of hormonal injections all the available methods such as those involving changes in light, temperature, pH, food, artificial aeration and kind and amount of vegetation were tried in vain. Also stripping and artificial fertilization were attempted unsuccessfully. This species was therefore finally omitted from the study. Each of the other nine species was kept in a separate aquarium, either in pairs or in schools as the nature of the species required. The dimension and the capacity of the aquarium was closely approximating that recommended by most aquarists. The room in which they were kept was fitted with a heater adjusted in cooler months to give a room temperature ranging from 75° to 85° F. In summer months the room temperature ascended as high as 93° F. but the temperature of the water never exceeded 84° F. Under even the highest temperatures running for a few days at a stretch, the fishes were apparently normal and healthy. Air pumps were used

only in summer months and only for nonlabyrinthine fishes or developing eggs and larvae, in order that there be sufficient oxygen for maintaining normal life and development. Filters made of plastic were used especially for aquaria containing nonlabyrinthine fishes; thus much change of water was not necessary.

The pH value, under laboratory conditions on occasional testing, ranged between 5.8 and 8.0. When the water in the aquarium became slightly cloudy and its pH value ran low, it was replaced by tap water that was allowed to stand for a time. This procedure for some reason not known was necessary for larvae and young fish, and it was, therefore, followed consistently for all aquaria. No attempt was made to maintain the pH value at a constant level. Considering the very low rate of mortality, and the success in breeding it may be said that these fishes are very hardy and readily acclimatized to laboratory conditions.

Aquatic plants such as fanwort and elodea were used sparingly except when they were needed for breeding purposes; they were arranged in the aquaria according to the need of the species. Though the anabantid fishes spawned without vegetation it was found by experience that rooted plants with their crown at the surface of the water helped to steady the bubble nest and gave harbor to the young; otherwise the young left in the aquarium would be eaten when the parents were hungry. In the case of Colisa lalia filamentous green algae and some debris were picked up by the male and thrown into the bubble nest, making the

nest firmer and more durable. In the absence of vegetation bubble nests were built by the anabantids in one of the corners of the aquarium. Hyphessobrycon serpae and Tanichthys albonubes required a nest made of fanwort or elodea weighted down in a shallow earthen bowl. This kind of nest prevented the parents from eating the eggs, which were collected at the bottom of the bowl and below the vegetation provided. Oryzias latipes laid eggs without plants but if plants were present the adhesive eggs became conveniently attached to them as the female swam about among them.

The remainder of the species, Brachydanio rerio, Brachydanio albolineatus and Brachydanio nigrofasciatus, spawned without vegetation. It was found necessary to induce spawning by artificial aeration and circulation of water, which was effected by allowing the air from a small pump (Thiberg Aerator, 2471796) to bubble slowly through a container in which the fish were placed. This container, which functioned as a 'nest', had its bottom covered with one or two layers of glass marbles in order that the parents which are carnivorous might not get at the eggs. When laid, the eggs fell in between the marbles to the bottom.

Food was given twice daily, once in the morning and once in the evening, the amount and time required for feeding being dependent on the size and number of the fish in a given aquarium. Both the prepared and live food were given, the former obtainable from a pet shop under the trade name 'Long Life', and the latter in the form of dophnids and brime shrimp which were reared in the laboratory. Also

when available mosquito larvae were fed. Occasionally chopped earthworms and hard boiled eggs were added to the diet. A small amount of food was always left at the bottom of the aquarium; therefore it was assumed that the quantity was adequate. The debris remaining after feeding was siphoned out daily. For the larvae at the early stages of development infusoria tablets were added and allowed to dissolve. This is actually inoculation with encysted or dried protozoa which become active in the water. For later stages brine shrimp larvae were used, in addition to the prepared food of micrograin size.

The nine species chosen for the work were from four families.

Family	
Characidae	Hyphessobrycon serpae Durban (Serpae)
	[illegible] (Serpae)
Cyprinidae	Brachydanio rerio (Hamilton=Buchanan) (Zebra Danio)
	Brachydanio albolineatus Blyth (Pearl Danio)
	Brachydanio nigrofasciatus (Day) (Spotted Danio)
	Tanichthys albonubes Lin (White Cloud Mountainfish)
Cyprinodontidae	Oryzias latipes (Temminck and Schlegel) (Geisha-Girl Medaka; Ricefish)
Anabantidae	Betta splendens Regan (Siamese Fighting Fish)
	Trichogaster trichopterus (Pallas) (Three-Spot or Blue Gourami)
	Colisa lalia (Hamilton=Buchanan) (Dwarf Gourami)

(Common name for each species is given in brackets below the scientific name).

Authorities disagree with regard to the assignment of the families to orders. Such differences of opinion generally result from the lack of uniformity in the weight or relative importance assigned to certain characteristics. The five best known classifications are given in Table I.

The general habits, spawning behavior, and embryonic development were observed; but the work was concentrated on the melanophores, their first appearance in the embryo, their early pattern formation, their movements, and their final and definitive positions before the adult coloration was acquired. The definitions of the arbitrary stages of development used are given below. All observations were recorded, and drawings were made with the help of the camera lucida.

Eggs of all species of Brachydanio were laid between five and seven o'clock in the morning in the nest prepared for them. The way the nest was made has been mentioned previously. After the marbles were removed with care the eggs were transferred by means of a large pipette to a finger bowl for cleaning and observation. If the fish are matured properly and fed well, eggs can be got almost every day.

The preparation of the nest for Hyphessobrycon serpae and Tanichthys albonubes has been described above. Both of these fishes spawn in schools. The removal of eggs of both species required great care as the eggs were sticky and attached to the bottom of the nest. At first the whole earthen bowl with the plants was lifted up from

TABLE I

Classification of the fishes according to recognized authorities on piscine taxonomy

Jordan (1923)	Regan (1929)	Berg (1940)	American Ichthyologists[2] (1950)	Axelrod and Schultz (1955)	Families	Genera and species
Heterognathi					Characidae	Hyphessobrycon serpae
	Osteriophysi	Cypriniformes	Cyprinida	Osteriophysoidei		
Eventognathi					Cyprinidae	Brachydanio rerio Brachydanio albolineatus Brachydanio nigrofasciatus Tanichthys albonubes
Cyprinodontes	Microcyprini	Cyprinodontiformes	Cyprinodontida	Cyprinodontoidei	Cyprinodontidae	Oryzias latipes
Percomorphi	Percomorphi	Perciformes	Percida	Percomorphoidei	Anabantidae	Betta splendens Trichogaster trichopterus Colisa lalia

[2]American Ichthyologists (1950) refers to the committee on fish classification of the American Society of Ichthyologists and Herpetologists, 1950

the aquarium and a little of the water was decanted. Then the plants were removed. On draining the water in the bowl slowly the eggs were exposed to view. A small amount of tap water that had been standing for a while was added to the bowl; thus the eggs could be washed free from the bottom. They were then transferred to a watch glass or a depression slide for observation.

Collection and observation of the eggs of bubble nest builders of the anabantid family was easy; they were either pipetted or scooped up with a finger bowl. However, care had to be taken in the case of the eggs of Betta splendens because the egg membrane is very fragile.

Oryzias latipes eggs were removed in two ways. The eggs were removed from the vegetation to which they became attached while the female carrying a bunch of eggs swam among the vegetation; or the eggs protruding from the genital opening of the female were removed by a brush. Generally, earlier stages of eggs were obtained by the second method and slightly later stages by the first method. The eggs were very hardy, as they were doubly protected by a tough egg-membrane and two kinds of threads external to it.

When batches contained more than ten eggs, ten were selected for observation; of batches containing less than ten eggs all eggs were used. At least four batches of each species were studied to determine ranges of variation in number of melanophores.

A few difficulties were encountered in the course of the observation and drawing. Some of the batches of eggs of Brachydanio rerio

had at times for reasons unknown a heavier animal pole which, therefore, stayed on the underside, thus keeping the embryo from view during observation under the microscope. Rolling and turning of these eggs did not help, as they were freely suspended in a comparatively large perivitelline space. Only those batches of eggs with the animal pole uppermost could thus be used for observation. A similar difficulty was encountered with the anabantid eggs that floated with the oil globule on the upper surface of the yolk; but these eggs could be rolled about in order to get the animal pole on the upper surface.

Drawing of the melanophores and of the outline of the advanced embryo presented another problem, as the periodic twitching of the body or even the slightest degree of flagellation of the tail caused a change in the position of the embryo before the completion of the drawing of a given view. Under the circumstances it became necessary to narcotize the embryo with a saturated menthol solution. This method allowed the animal to remain stationary for about ten minutes. After that it died, possibly due to poisoning. If, at the end of ten minutes, it was transferred to water it revived. However, in many instances more than ten minutes were required to complete the drawing of the melanophores in detail. In such a case the animal was sacrificed, thus necessitating using another embryo of the same stage and batch, or of a similar stage of the next batch.

The last technical difficulty that arose in this work was inevitable; to observe even ten embryos of the same stage at about the

same time for counting or examining the melanophores proved especially difficult in the case of the quickly developing embryos such as those of Colisa lalia. Hence the only practicable way was to fix the other nine embryos of the same stage either in 75% ethyl alcohol or in 5% formalin and examine them within a few days before the lighter melanophores became too faded to be used. As a consequence one needed ten times as many eggs as there were stages to be studied. That is, for the four arbitrary stages of melanophore formation a minimum of 40 eggs was required. Actually a minimum of 160 embryos was required to complete the study of each species. However, the demand was well met because the fishes studied were highly prolific. Those that had fewer eggs in a batch spawned repeatedly within a few days' interval; and those that spawned at longer intervals had a large number of eggs in a batch; and finally there were those that spawned often with many eggs at a time.

Egg measurements were taken in terms of total overall size inclusive of all membranes rather than of the living undivided cell only. This method might be expected to show features beyond the variable of cell shrinkage consequent upon fertilization.

TABLE II

Spawning records and number of eggs studied

Species	Method of obtaining eggs	Spawning time[3]	Date of Spawning	No. of eggs in each batch
Hyphessobrycon serpae	From Schools	5 a.m. to 7 a.m.	Feb. 26, 1956	56
			Mar. 2, 1956	78
			May 6, 1956	183
			May 16, 1956	23
			June 2, 1956	63
Brachydanio rerio		5 a.m. to 7 a.m.	Nov. 20, 1954	7
			Nov. 22, 1954	54
			Dec. 6, 1954	85
			Dec. 13, 1954	115
			Dec. 21, 1954	54
			Jan. 7, 1955	62
Brachydanio albolineatus		5 a.m. to 7 a.m.	Nov. 23, 1954	88
			Dec. 2, 1954	101
			Dec. 6, 1954	162
			Dec. 13, 1954	43
			Dec. 19, 1954	94
			Mar. 4, 1956	156
Brachydanio nigrofasciatus		5 a.m. to 7 a.m.	June 23, 1955	40
			June 25, 1955	55
			June 27, 1955	33
			June 28, 1955	128

TABLE II CONT'D.

Species	Method of obtaining eggs	Spawning time[3]	Date of Spawning	No. of eggs in each batch
Tanichthys albonubes	)	5 am.m. to 7 a.m.	Oct. 9, 1955	64
	)		Oct. 10, 1955	14
	)		Oct. 12, 1955	11
	)		Oct. 17, 1955	18
	)		Oct. 18, 1955	40
	)		Oct. 19, 1955	10
	)		Oct. 26, 1955	140
	)		Oct. 27, 1955	52
Oryzias latipes	)	Irregular	Dec. 27, 1955	5
	) From		Dec. 28, 1955	13
	) Schools		Dec. 29, 1955	11
			Dec. 30, 1955	8
			Dec. 31, 1955	18
			Jan. 1, 1956	13
			Jan. 2, 1956	12
			Jan. 3, 1956	9
			Jan. 4, 1956	4
			Jan. 5, 1956	9
			Jan. 6, 1956	26
			Jan. 7, 1956	14
			Jan. 9, 1956	15
			Jan. 10, 1956	28

TABLE II CONT'D.

Species	Method of obtaining eggs	Spawning time[3]	Date of Spawning	No. of eggs in each batch
Betta splendens	From pairs	Irregular	Nov. 7, 1954	126
			Dec. 27, 1954	128
			June 10, 1955	132
			June 18, 1955	138
Trichogaster trichopterus		Irregular	Dec. 17, 1954	242
			Dec. 27, 1954	53
			May 16, 1955	96
			Oct. 26, 1955	140
			Nov. 14, 1955	58
Colisa lalia		Irregular	Nov. 14, 1955	26
			Nov. 17, 1955	33
			Mar. 29, 1956	220
			Apr. 27, 1956	214
			May 14, 1956	85

[3]This table merely records the date of spawning of fishes studied. No attempt is made to cover the possible breeding season of each species.

SPECIES CHARACTERISTICS EXCLUSIVE OF PIGMENTATION

FERTILIZED EGG. The egg of Hyphessobrycon serpae ranges in diameter from 0.99 mm. to 1.15 mm. and has an average diameter of 1.07 mm. It is round, slightly opaque, and tinged with pink. The perivitelline space, which is fairly large, measures on the average 0.21 mm. The egg is heavier than water, without oil globules and with fine yolk granules. The animal pole assumes no definite position. The egg-membrane is thin, highly fragile, and fairly adhesive. This membrane is provided with a con-like dent on one side with its apex turned towards the center of the egg. From this dent radiate a number of grooved lines branched and unbranched towards the equator from which they continue to the opposite pole, where they converge. The dent with its shape and structure is highly suggestive of the micropyle found in some fish eggs such as those described by Mookerjee and Mazumdar (1946b) in Notopterus notopterus (Pallas). The grooved lines of the egg-membrane become wider and less prominent as development proceeds, until finally they disappear altogether. The dent mentioned above, however, persists until hatching. It would be interesting to determine the function of the dent and of the grooved lines of the egg membrane in this fish.

The egg of Brachydanio rerio measures between 1.21 mm. and 1.29 mm. in diameter, with an average of 1.27 mm. It is round, colorless

and nearly opaque. The large perivitelline space measures on the average 0.31 mm. The egg is heavier than water, without oil globules and with fine yolk granules. The animal pole takes no particular position except that in two batches of eggs the animal pole was found to be persistently on the under side. The egg-membrane is smooth, nonadhesive and tough. There are no specific characteristics on the egg-membrane.

The egg of Brachydanio albolineatus is round, colorless and nearly transparent, ranging in diameter from 1.37 mm. to 1.58 mm. and measuring on the average 1.44 mm. It has a very large perivitelline space with an average measurement of 0.34 mm. There are no oil globules, the yolk granules are fine, and since the egg is heavier than water it rests on the bottom. The egg-membrane is smooth, nonadhesive, tough and free from surface modification.

The egg of Brachydanio nigrofasciatus is round, colorless, and nearly transparent. Its diameter ranges from 1.20 mm. to 1.42 mm., with an average of 1.31 mm. The large perivitelline space measures 0.34 mm. As is the case in the eggs of the other two Brachydanios, there are no oil globules and the yolk granules are fine. This egg is also heavier than water. The egg-membrane is smooth, tough, nonadhesive and free from surface modification.

The egg of Tanichthys albonubes is round, tinged with yellow, and slightly opaque. The diameter of the egg measures between 1.07 mm. and 1.23 mm., and has an average measurement of 1.15 mm. It has a

fairly large perivitelline space of about 0.13 mm. The egg is provided with no oil globules, but has fine yolk granules, and is heavier than water. The egg-membrane is fairly strong, smooth, slightly adhesive, and without surface modification.

The egg of Oryzias latipes is round, slightly opaque and whitish. Its diameter measures from 1.27 mm. to 1.30 mm., and has the average of 1.28 mm. It has a very small perivitelline space, measuring only 0.07 mm. The yolk grains are coarse and there are from a few to many oil globules. The size of the oil globules varies inversely with the number, that is, when the number is small the globules are larger and when the number is large the oil globules are smaller, thus probably keeping a constant total volume. The egg-membrane is thick, and tough due to the presence of two kinds of chorionic filaments. Of these the shorter and stouter ones are widely scattered all over the surface except at the vegetal pole, and each is about 0.025 mm. long, fairly quickly tapering and terminally crooked or wavy. The other type of thread, confined to the vegetal pole, is fine, whitish in color, 0.95 mm. long on the average and sticky, capable of being stretched to nearly twice the original length. By these two kinds of filaments the eggs are entangled together or attached to the debris or vegetation individually or in a mass. It may be observed that the oil globules coalesce as development proceeds, till in the advanced stage only one large one is left, and this disappears with the absorption of the yolk. The oil globules are located in the vegetal

hemisphere. A single large oil globule is found in the advanced embryo either to the left or the right of the snout. Rugh (1952) states that the function of the oil globule is nutritive. It is remarkable that these oil globules so commonly found in the pelagic fish eggs are also found in this highly demersal egg.

The egg of Betta splendens, unlike any other egg mentioned so far, is without a fixed shape at fertilization. This is probably because its membrane, highly fragile, much wrinkled and thin, is not fully stretched at this stage. The side on which it rests or by which it is in contact with an object becomes either flat or indented. When it is fully distended, it is oval or round, measuring on the average 1.13 mm. and ranging from 1.07 mm. to 1.15 mm. The egg is whitish, opaque, and has coarse yolk granules. Unlike other anabantid eggs, it is not provided with oil globules for flotation and being heavier than water sinks to the bottom. The male then picks up the eggs as they sink and throws them back into the bubble nest. The egg-membrane mentioned previously has a large number of branched and unbranched wrinkled lines that have no special arrangement. These wrinkles are in the form of grooved lines that become broader and less distinct as development progresses. Whether they serve to allow the egg-membrane to stretch by unwrinkling is not known.

The egg of Trichogaster trichopterus is round, whitish, and slightly opaque, its diameter ranging between 0.69 mm. and 0.96 mm. with an average of 0.83 mm. The yolk grains are coarse and the egg is provided

with a single large oil globule that serves to float the egg even without the help of external support such as the air bubbles of the nest. The animal pole under normal conditions always occupies the under side in the free egg, and the oil globule on the vegetal pole, therefore, lies on the upper surface. The tough egg-membrane, except for very faint wrinkles, may be described as smooth. A cone-shaped dent, found at one side of the egg-membrane and persisting till gastrulation, has its apex pointed toward the center of the egg. As has been suggested for other fish (Mookerjee and Mazumdar 1946b) this may be interpreted as the point of entry of the sperm.

The egg of Colisa lalia, like that of Trichogaster trichopterus, is round, whitish, slightly opaque, and provided with a large single oil globule and coarse yolk grains. It is also able to float without the help of the air bubbles of the nest. It has a diameter range of 0.76 mm. to 0.80 mm., with an average of 0.78 mm. Its small perivitelline space measures 0.06 mm. Like the egg of Trichogaster trichopterus, its animal pole is on the under side and the vegetal pole containing the oil globule is on the upper side. The membrane of the egg is tough, nonadhesive, its wrinkles few and barely visible. It is also noteworthy that there is in every egg a conelike dent in the membrane with its apex directed to the interior of the egg. Its possible function has been suggested above.

Attention is called to the fact that as will be pointed out later none of the features described above appear taxonomically significant.

TABLE III STRUCTURE OF THE FERTILIZED EGG

Column 9 For materials as to the oil globules of Oryzias latipes, see below discussion

Column 10 "Sink" or "float" refers respectively to the inherent tendency to fall to the bottom because it is heavier than water or to float because it is lighter than water

Column 11 "Adhesiveness" here refers to the stickiness of the general surface of the membrane rather than to the stickiness of the accessory structures such as chorionic filaments

(1) Species	(2) Shape	(3) Color	(4) Transparency	(5) Diameter range	(6) Average Diameter	(7) Perivitelline space	(8) Yolk granules	(9) Oil globules	(10) Buoyancy	(11) Position of animal poles	(12) Adhesive or non-adhesive	(13) Free or attached	(14) Surface of egg-membrane	(15) Comparative toughness of egg-membrane
Hyphessobrycon serpae	Round	Pinkish	Slightly opaque	0.99mm.-1.15mm.	1.07 mm.	0.01 mm. Fairly large	Fine	Absent	Sinks	Variable	Slightly adhesive	Attached to bottom	Strongly wrinkled A cone-like dent	Highly fragile
Brachydanio rerio	Round	Color-less	Nearly transparent	1.21mm.-1.29mm.	1.27 mm.	0.11 mm. Large	Fine	Absent	Sinks	Variable	Non-adhesive	Free	No wrinkles Smooth	Tough
Brachydanio albolineatus	Round	Color-less	Nearly transparent	1.37mm.-1.53mm.	1.41 mm.	0.34 mm. Very Large	Fine	Absent	Sinks	Variable	Non-adhesive	Free	No wrinkles Smooth	Tough
Brachydanio nigrofasciatus	Round	Color-less	Nearly transparent	1.20mm.-1.42mm.	1.31 mm.	0.34 mm. Very large	Fine	Absent	Sinks	Variable	Non-adhesive	Free	No wrinkles Smooth	Tough
Tanichthys albonubes	Round	Yellow tinge	Slightly opaque	1.07mm.-1.23mm.	1.15 mm.	0.13 mm. Fairly small	Fine	Absent	Sinks	Variable	Slightly adhesive	Attached to bottom	No wrinkles Smooth	Less tough
Oryzias latipes	Round	Slightly whitish	Fairly opaque	1.27mm.-1.30mm.	1.28 mm.	0.07 mm. Very small	Coarse	Few to many	Sinks	Variable	Non-adhesive	Attached to vegetation	Rough with two kinds of filaments	Very tough
Betta splendens	Oval	Whitish	Opaque	1.07mm.-1.15mm.	1.13 mm.	0.19 mm. Fairly small	Coarse	Absent	Sinks	Variable	Non-adhesive	Attached to bubble nest	Strongly wrinkled	Highly fragile
Trichogaster trichopterus	Round	Slightly whitish	Slightly opaque	0.69mm.-0.96mm.	0.83 mm.	0.15 mm. Small	Coarse	Single large	Floats	Under side	Non-adhesive	Attached to bubble nest	Faintly wrinkled A cone-like dent	Tough
Colisa lalia	Round	Slightly whitish	Slightly opaque	0.76mm.-0.80mm.	0.78 mm.	0.06 mm. Very small	Coarse	Single large	Floats	Under side	Non-adhesive	Attached to bubble nest	Faintly wrinkled A cone-like dent	Tough

BLASTULA. The discoblastula, which is characteristic of teleost fishes, is found here to vary in shape. In one type it is a low disc in which the base is broader than its thickness. In another type it is hemispherical, with the base as broad as the thickness of the disc. In the third type the blastodic is an almost perfect sphere, connected to the yolk mass by a narrowed junction.

The time interval taken to complete blastulation varies within a narrow range among the species, with the exception that the developmental rate of Oryzias latipes is much slower than that of any other species.

TABLE IV

Approximate time required to complete blastulation, with shape of fully formed blastula

Species	Time required to complete blastulation	Shape of fully formed blastula
Hyphessobrycon serpae	4.00 hrs.	Low disc
Brachydanio rerio	4.50 hrs.	Hemispherical
Brachydanio albolineatus	4.00 hrs.	Low disc
Brachydanio nigrofasciatus	3.75 hrs.	Low disc
Tanichthys albomubes	5.00 hrs.	Low disc
Oryzias latipes	6.00-8.00 hrs.	Hemispherical
Betta splendens	4.00 hrs.	Nearly spherical
Trichogaster trichopterus	5.00-5.50 hrs.	Low disc
Colisa lalia	3.50 hrs.	Low disc

EMBRYONIC SHIELD. The time required for the first appearance of the embryonic shield varies only slightly among the individuals of a batch. It is impossible to keep the different batches of eggs of the same species under identical environmental conditions; temperature, oxygen, and fungus growth on the eggs are the main factors responsible for a wide variation in time, even among the batches of the same species. On the other hand the position of the germ ring on the yolk and the amount of yolk covered by the germ disc at the time when the embryonic shield first appears is identical in all the individuals even of different batches of the same species.

TABLE V

Time interval required for first appearance of embryonic shield, and amount of yolk covered by germ disc when shield first appears

Species	Average time of first appearance after laying	Position of germ ring when shield first appears	Amount of yolk covered by germ disc
Hyphessobrycon serpae	6.00 hours	Well beyond equator of egg	3/4 approximately
Brachydanio rerio	14.00 hours	Just beyond equator of egg	Slightly more than 1/2
Brachydanio albolineatus	12.00 hours	Far beyond equator of egg	5/8 approximately
Brachydanio nigrofasciatus	12.00 hours	Far beyond equator of egg	5/8 approximately
Tanichthys albonubes	16.00 hours	Beyond equator of egg	2/3 approximately
Oryzias latipes	17.00 hours	At equator of egg	1/2 approximately
Betta splendens	4.50 hours	Beyond equator of egg	2/3 approximately
Trichogaster trichopterus	7.00 hours	Beyond equator of egg	Less than 2/3
Colisa lalia	6.00 hours	Beyond equator of egg	2/3 approximately

PRE-PIGMENT STAGE. In the pre-pigment stage circulation has started in Brachydanio rerio, Brachydanio albolineatus, Brachydanio nigrofasciatus, Tanichthys albonubes and Oryzias latipes; but not in Hyphessobrycon serpae, Betta splendens, Trichogaster trichopterus and Colisa lalia. In these latter fishes it is found that the somites are comparatively fewer and organs such as eye, ear and tail are less developed. It is noted that in the early stage the eye is oval but as development progresses it becomes round and fitted with lens.

TABLE VI

Pre-pigment stage showing significant differences in early organogeny

Species	Hours	Somites	Tail	Eye	Ear	Kuppfer's Vesicle	Circulation	Shape of yolk seen from side view
Hyphessobrycon serpae	16.33	20-22	Free, bent, compressed	Oval, without lens	No otoliths	Absent	Not started	Oval
Brachydanio rerio	30.00-35.00	29-30	Free, bent at an angle, compressed	Round, with lens fitted	Two otoliths	Absent	Started	Oval
Brachydanio albolineatus	22.00-28.00	20-23	Free, bent at an angle, compressed	Round, with lens fitted	Two otoliths	Absent	Started	Oval
Brachydanio nigrofasciatus	47.00	29-30	Free, bent at an angle, compressed	Round, with lens fitted	Two otoliths	Absent	Started	Oval
Tanichthys albonubes	20.50	38-40	Free, curved round yolk, compressed	Round, with lens fitted	Two otoliths	Absent	Started	Oval
Oryzias latipes	44.00	17-18	Not free, depressed	Oval, with lens nearly fitted	No otoliths	Present	Started	Round
Betta splendens	20.00-30.00	14-18	Only tip free, round	Oval, with lens nearly fitted	No otoliths	Present	Not started	Round
Trichogaster trichopterus	14.75	12-15	Not free, depressed	Oval, with lens nearly fitted	No otoliths	Present	Not started	Round
Colisa lalia	24.00	13-17	Not free, depressed	Oval, lens not formed	Not formed yet	Present	Not started	Round

EMBRYO AT HATCHING. At hatching all embryos are alike in general shape and structure except Hyphessobrycon serpae and Betta splendens which are provided with embryonic adhesive structures. In Hyphessobrycon serpae this consists of a disc on the head. In Betta splendens it takes the form of a number of papillae on the head and nape.

Young of Hyphessobrycon serpae at hatching are poorly developed; they are without visible melanophores or circulation. Though hatched at a very undeveloped stage, larvae seem to differentiate and grow normally after hatching; unfortunately none were reared to adulthood. The method of hatching in all these fishes is the same. When the membrane breaks the embryo emerges tail first.

TABLE VII

Embryo at hatching

Species	Somites	Time of hatching	Size and shape of yolk seen from side	Peculiar embryonic structures
Hyphessobrycon serpae	37-38	38.33 hrs.	Large, oval	Disc on head
Brachydanio rerio	33-34	78.00 hrs.	Large, oval	None
Brachydanio albolineatus	32-33	73.00 hrs.	Large, oval	None
Brachydanio nigrofasciatus	30-34	50.00 hrs.	Small, elongate	None
Tanichythys albonubes	30-32	48.00-50.00 hrs.	Small, elongate	None
Oryzias latipes	30-31	144.00-288.00 hrs.	Small, elongate	None
Betta splendens	25-29	50.00 hrs.	Large, oval	Papillae on head and nape
Trichogaster trichopterus	29-30	20.00 hrs.	Large, oval	None
Colisa lalia	24-25	19.00-20.00 hrs.	Large, oval	None

PIGMENTATION

This paper deals primarily with melanophores. The chemistry of melanin and its relation to other pigments is not considered herein. The period to be analyzed is the interval between the first occurrence of melanophores and the final establishment of a stable pigment pattern either before or after hatching. For purposes of comparison of the pigment formation among the different species it is convenient to divide the above period into four arbitrary stages: (1) initial stage, (2) primary stage, (3) transitional stage, and (4) final stage. These stages are not static but one merges into the other at varying speeds.

(1) The initial stage is recognized by the first appearance of the melanophores in any region of the body of the embryo.

(2) The primary stage follows the initial stage; it is distinguished by the formation of a sort of pattern peculiar to the embryo of a given species.

(3) The transitional stage follows the primary stage; it is marked by the presence of a large number of melanophores moving and spreading backward either diagonally or axially. It is not referable to any special pattern; the positions of most of the melanophores constantly shift.

(4) The final stage follows the transitional stage. It is recognized by the orderly arrangement of melanophores into a characteristic pattern that is stable for some time before the adult coloration is established.

INITIAL STAGE OF PIGMENTATION

The eye is the initial site of pigmentation in the embryos of Cyprinidae and Cyprinodontidae (Figs. 2-6). Very fine granules of dark brown color first appear in the eye region. These granules are much fewer at this stage and concentrated about the antero-dorsal part of the eye. The area of the eye near the choroid fissure is free from such granules. All members of Cyprinidae during the initial stage of pigmentation are at nearly the same stage of embryonic development, with fully formed eye and ear, pulsating heart and free tail. The number of somites and the time taken to arrive at this stage are also similar. On the other hand the embryo of Oryzias latipes (Cyprinodontidae) is less advanced in development, taking 54 hours after fertilization to form comparatively fewer somites and less developed eye and ear. The tail also is only partly free.

In Hyphessobrycon serpae (Characidae) the initial site of pigmentation includes the nape, yolk sac and anal and post-anal regions (Fig. 1). Though nineteen to twenty somites are already formed within a comparatively short interval of 14 to 16 hours, the embryo is not far advanced in the development of the eye and the ear with their component parts. There is no visible pulsation of the heart, nor is the blood visibly circulating.

Members of Anabantidae have their initial site of pigmentation on the yolk sac (Figs. 7-9). These embryos, at this stage, are far

less advanced in development than those of any other species. The eye is either without lens or with lens (Betta splendens) in very early stage, and the ear is always sac-like and without any indication of otoliths. There is no pulsation of the heart, and the tail, if free, is only partly so and is closely adherent to the round yolk.

A comparative account of the embryos at the initial stage of pigmentation, as shown in Table VIII, indicates that members of the same family have the same site of initial pigmentation and that they are nearly at the same stage of embryonic development. This is true in the case of the families Cyprinidae and Anabantidae. Members of these two families, however, have very different rates of embryonic development, each characteristic of all the genera and species in that family. Different families with the same site of initial pigmentation (Cyprinidae and Cyprinodontidae) are not characterized by like embryonic developmental rate; the embryos in Cyprinidae are far advanced over those of Cyprinodontidae, and the times taken to reach this stage are also very different.

TABLE VIII

Initial stage of pigmentation with reference to site (or areas) of pigmentation and parallel degree of development of embryo

Species	Pigmented areas	No. of Somites	Average time after fertilization	Eye	Ear	Movement of heart	Circulation	Pectoral bud	Kuppfer's vesicle	Tail	Yolk
Characidae											
Hyphessobrycon serpae	Nape, yolk sac, anal, post anal	19-20	15	Oval, with no lens	No otoliths	-	-	-	+	Free	Oval
Cyprinidae											
Brachydanio rerio	Eye	29-30	17	Rounded, with lens	Otoliths	+	+	+	-	Free	Oval
Brachydanio albolineatus	Eye	20-23	21	Rounded, with lens	Otoliths	+	+	+	-	Free	Oval
Brachydanio nigrofasciatus	Eye	29-30	25	Rounded, with lens	Otoliths	+	+	+	-	Free	Oval
Tanichthys albonubes	Eye	27-28	15	Rounded, with lens	Otoliths	+	+	-	-	Free	Oval
Cyprinodontidae											
Oryzias latipes	Eye	17-19	54	Rounded, with lens	No otoliths	+	+	-	-	Partly free	Round
Anabantidae											
Betta splendens	Yolk sac	17-18	24	Oval, with lens	No otoliths	-	-	+	-	Only tip free	Round
Trichogaster trichopterus	Yolk sac	12-13	15	Oval, with no lens	No otoliths	-	-	-	-	Nearly free	Round
Colisa lalia	Yolk sac	13-15	14	Oval, with no lens	No otoliths	-	-	-	+	Not free	Round

PRIMARY STAGE OF PIGMENTATION

At this stage melanophores have appeared on other areas of the body of the embryo with some increase in quantity in the respective initial sites of their formation. With the exception of those in the eye[4], where they are non-stellate and black, the melanophores are of stellate type with few arms and dark brown color. Recognizable differences exist between the species at this stage, the recognition being dependent not only on the key areas in which the melanophores are present but also on the number and manner of their distribution in those areas that overlap between species. A pattern characteristic of each species is recognizable. As defined above this primary stage of pigmentation remains stable for a certain time before merging into the next stage.

Hyphessobrycon serpae like the anabantids has no melanophores in the eye at this stage, but unlike the latter it has melanophores in the tail region. In fact this is the only species that has melanophores in the tail region at this stage. (Table IX and Fig. 10). Besides, it may be recalled that, in the previous stage, the initial site of pigmentation is entirely different in Hyphessobrycon serpae from that in the anabantids; in the former case the initial site includes nape, yolk sac, anal and post-anal regions, whereas in the

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[4]As it is impossible to count the pigment granules in the eye, the number of melanophores of this region is not given in Table IX.

latter case it includes only the yolk sac.

As noted before, the anabantids are unique in having the yolk sac as the initial site of pigmentation. In this stage the pigmentation spreads into the same region in all species and is similarly distributed with the single exception that in Colisa lalia there are additional areas developed in the head. The melanophores of Trichogaster trichopterus and Colisa lalia are larger than those of Betta splendens (Table IX and Figs. 16-18). In the family Anabantidae there are definite family characters not met with in any of the other families studied; such are melanophores on nape, auditory region, trunk and yolk sac.

A group in which the eye forms the initial site of pigmentation is made up of two families, Cyprinidae and Cyprinodontidae. The eyes in these are darker at this stage than in the previous stage. The two families can at this stage be separated by the manner of distribution of melanophores on the yolk sac (Figs. 11-15). In the members of the cyprinid family the melanophores are either absent on the yolk sac (Tanichthys albonubes) or when present are restricted to the dorso-lateral region of the yolk sac (Brachydanio rerio, Brachydanio albolineatus and Brachydanio nigrofasciatus). On the other hand the melanophores on the yolk sac of Oryzias latipes (Cyprinodontidae) are widely scattered.

The three species of Brachydanio are identical in the key areas of melanophore formation but differ from one another perceptibly in

the manner of distribution in these areas as well as in the total count of melanophores in certain areas. The two genera of Cyprinidae (Brachydanio and Tanichthys) differ widely in the key areas of melanophore formation; the difference is seen on the trunk and yolk sac as shown in Table IX and Figures 10 to 13.

Oryzias latipes (Cyprinodontidae) except for the trunk has similar key areas of melanophore formation as the Brachydanios, which differ in this regard from Tanichthys. But melanophores on the yolk sac of Oryzias latipes are widely scattered all over, as stated before; this is not so with Tanichthys albonubes nor with the Brachydanios.

Considering the melanophore formation at this stage on the basis of key areas, number and manner of distribution, it is found that similarities are greater between any of the species within a given genus than between any of the species and those of a different genus. As to similarities between genera of one family in comparison with those of another family, the data from these two families are inconclusive.

TABLE IX[5]

Comparison of distribution of melanophores on key areas at the primary stage of pigmentation

Species	Eye	Head	Nape and auditory region	Trunk	Tail	Yolk sac	Total
Characidae							
Hyphessobrycon serpae	-	0	8	0	11	44	63
Cyprinidae							
Brachydanio rerio	+	2	11	20	0	5	38
Brachydanio albolineatus	+	2	15	10	0	9	36
Brachydanio nigrofasciatus	+	2	16	5	0	8	31
Tanichthys albonubes	+	3	4	0	0	0	7
Cyprinodontidae							
Oryzias latipes	+	8	4	0	0	8	20
Anabantidae							
Betta splendens	-	0	2	4	0	16	22
Trichogaster trichopterus	-	0	2	3	0	29	34
Colisa lalia	-	3	3	2	0	15	23

[5]Attention is called to the fact that the count is made only on one side (left) of the body and that the number is the average of ten counts.

TRANSITIONAL STAGE OF PIGMENTATION

In this stage the melanophores have increased considerably in number, especially in the posterior part of the trunk and the tail in most of the species. The eye is pigmented in all the species. This stage is also recognized by the posteriorly directed movement of the melanophores either axially or diagonally downward, the movement being more clearly noticeable in the mid-trunk and the basal region of the tail and in some cases on the yolk sac. It is observed that these moving melanophores are stretched into thin outline in the direction of their movement. During this period of transition the stretched melanophores are brownish in color in contrast to the others which appear darker and somewhat thicker in outline. This may be due to the spread of granules in the stretched ones in contrast to the close packing of granules in the contracted ones. The overall effects result in the general spreading posteriorly and downward (Figs. 19-27). The transitional stage of pigmentation is completed before hatching in Cyprinidae but the features characteristic of the transitional stage are extended to the early larval stage in Characidae, Cyprinodontidae and Anabantidae. There are recognizable differences between the families with regard to nature, arrangement and number of melanophores. In the family Characidae as represented here by Hyphessobrycon serpae the melanophores of the yolk sac give a highly netted appearance by the anastomosing of

their processes. This is not noticeable in any of the other families studied. Likewise, Hyphessobrycon serpae, unlike members of the other families studied, is marked by the entire absence of melanophores in the lateral and dorsal region between the eye and the pectoral fin.

In the species of Cyprinidae the melanophores of this stage are more or less evenly distributed from the eye nearly to the tip of the tail, the melanophores being fewest in Tanichthys albonubes and most in Brachydanio nigrofasciatus. The absence of melanophores at the antero-ventral side of the yolk sac is also observed in this group.

In Oryzias latipes (Cyprinodontidae) the melanophores concentrate on the dorsal side of the head and the nape. In the trunk and tail they are arranged in two lines, one on either side of the mid-dorsal line; and on the yolk sac they are comparatively few and widely scattered.

In the anabantids the yolk sac has a large number of melanophores fairly evenly distributed, which fact alone distinguishes the members of this family from those of other families. Betta splendens is different from Trichogaster trichopterus and Colisa lalia. At this stage the tail region of Betta splendens is devoid of melanophores; in the other two the melanophores on the tail are arranged almost segmentally, that is, they appear to form rows between the myotomes.

At this stage no generalizations based on melanophores can be made regarding the relationship of the families, genera or species, as the melanophores are still in the process of moving.

FINAL STAGE OF PIGMENTATION

This is the stage which follows the transitional stage. The melanophores which are larger, fewer and darker than in the previous stage establish themselves in definitive positions, giving a characteristic pattern peculiar to the embryos of the species. This pattern is stable for a certain length of time before the adult coloration is acquired. In members of the family Cyprinidae the final stage of pigmentation is initiated and established before hatching. In the remainder of the families studied it is initiated and completed after hatching. As this stage of pigmentation is stable and as the pattern formed is peculiar to the species, this stage may be considered as important for identification, as well as for the determination of the affinities between the species, genera and families. But the consideration of this stage alone may not be sufficient evidence of relationship within families; other important characters stressed in earlier stages must be taken into account.

For a comparative study of melanophore formation of this stage some areas are arbitrarily selected. These areas are the head, the nape, the yolk sac, the primitive intestine, the trunk and the tail. The melanophores of the trunk and tail region are arranged in three linear series which are named according to position dorso-lateral, mid-lateral and ventro-lateral series or lines. For our present

purposes the eye, fully pigmented at this stage, and the deeper melanophores such as those of the peritoneum, masked to a varying extent by the dermal melanophores, are not taken into account. That is, the extra-ocular and dermal melanophores are the main ones used here for the purposes of comparison. A quantitative consideration of the melanophores of this stage is given in Table X.

In the family Cyprinidae (two genera and four species) the three species of Brachydanio show recognizable differences regarding the detailed arrangement of melanophores, though otherwise they are very similar; the number of melanophores in these two regions are approximately the same (from 14-19) and they are distributed in similar manner. The only noticeable difference in these two regions is that in Brachydanio albolineatus there are always 3 to 4 melanophores below the auditory organ, which is not the case in the other two species of Brachydanio.

The yolk sac and the primitive intestinal region show another noticeable difference; there are 60 melanophores in Brachydanio nigrofasciatus in these regions taken together, whereas in the other two the number is much smaller, ranging from 30 to 31. The distribution of melanophores on the yolk sac is also different. In Brachydanio nigrofasciatus the melanophores are evenly distributed. In Brachydanio albolineatus there are melanophores on the ventral side of the yolk sac; but this is not in the case of Brachydanio rerio, in which the anterior ones form a vertical line that is distinctive of the species.

The dorso-lateral series of melanophores extends from the pectoral fin to the tip of the tail vertebrae. These melanophores are of stellate shape, the anterior ones being slightly larger than the posterior. The number of melanophores are respectively 35, 32 and 36 in Brachydanio rerio, Brachydanio albolineatus and Brachydanio nigrofasciatus. The dorso-lateral series that lies close to the mid-dorsal line appears quite prominent in all the three species, and this line is alike in all.

In the mid-lateral series of melanophores there are distinguishable differences among the species of Brachydanio. This series runs from the pectoral region above the yolk sac to the tip of the tail and lies parallel with the dorso-lateral series. The number of melanophores forming this series is 17 in Brachydanio rerio, 10 in Brachydanio albolineatus, and 16 in Brachydanio nigrofasciatus. In all the species is series is interrupted, but the way in which it is interrupted differs. In Brachydanio rerio the line begins from the pectoral fin, is broken above the posterior part of the primitive intestine, and continues from the basal third of the tail to nearly the tip of the tail vertebrae. In Brachydanio albolineatus this series begins from behind the yolk sac region and extends halfway to the last of the tail vertebrae, the rest of the tail being devoid of melanophores. In Brachydanio nigrofasciatus this series begins as in Brachydanio albolineatus, but in the rest of the line the melanophores are arranged as are those of Brychydanio rerio.

The last linear series of melanophores, the ventro-lateral line, stretches from behind the gut to the last of the tail vertebrae, the number of melanophores being 20, 19 and 22 respectively in the species in the above order. This series is nearly double at about the level of the anus but single behind that. Except for the slight interruption at the tail tip in Brachydanio albolineatus the series is very much the same in all.

Of the three species of Brachodanio, Brachydanio nigrofasciatus is the darkest, and Brachydanio albolineatus is the lightest. This variation in shade is due to the total number of melanophores present in each of them, the number being 117 in Brachydanio rerio, 111 in Brachydanio albolineatus and 148 in Brachydanio nigrofasciatus.

The pigmentation of Tanichthys albonubes, another member of family Cyprinidae, resembles that of species of Brachydanio. There are 19 melanophores in the head and nape regions, 24 in the yolk sac and gut regions, 23 in the dorso-lateral series, 12 in the mid-lateral series, and 27 in the ventro-lateral series, thus making a total of 105 melanophores. Unlike the condition in the species of Brachydanio the region in front of the eye is devoid of melanophores. The yolk sac and the gut regions together have a characteristic arrangement of melanophores different from that in the species of Brachydanio. Anteriorly the yolk sac has 3 melanophores arranged vertically in a row. A little behind this the melanophores form two lines, one in front in vertical direction and the other behind in horizontal

direction. These two lines meet at an angle t give the appearance of the letter L.

Oryzias latipes reaches the final stage of pigmentation after hatching. The number of melanophores are 13 in the head and nape region, 15 in the yolk sac and gut region, 28 in the dorso-lateral line, 20 in the mid-lateral line, and 20 in the ventro-lateral line, forming altogether a total of 96 melanophores. The total number of melanophores is fewer than those of the members of the Cyprinidae. The melanophores on the dorsal side of the head are very large, and, being supplied with very short arms, appear angular in outline. This type of melanophore is called the corolla-type by Tavolga (1949). The snout is free from melanophores as in Tanichthys albonubes; those of the yolk are without recognizable pattern. Of the three linear series of melanophores only the mid-lateral series is incomplete; this series begins somewhat behind the pectoral fin and ends well before the last of the tail vertebrae.

The members of the family Anabantidae, represented by our three genera (Betta splendens, Trichogaster trichopterus and Colisa lalia), reach the final stage of pigmentation only after hatching. The head and the nape regions together have approximately the same number of melanophores, the number being respectively 14, 16 and 12 in the above order. The yolk sac and the gut regions show 27 in Betta, 30 in Trichogaster, and 45 in Colisa. The dorso-lateral line is absent altogether in Betta, complete in Trichogaster, and stops short of

the end of the tail in Colisa. The numbers of melanophores in this series are 0, 32 and 28 respectively in the above order. The mid-ventral line is absent again in Betta, incomplete at the two terminals in Trichogaster, and stops short of the posterior one-third of the tail in Colisa. The number of melanophores is 0 in Betta, 21 in Trichogaster, and 22 in Colisa. The ventro-lateral line is made up of 16 melanophores in Betta, 21 in Trichogaster, and 24 in Colisa. This line is shortest or least developed in Betta, represented by the anterior part only. In Trichogaster it is the most developed and complete, reaching the end of the tail. In Colisa it stops short of the posterior one-third of the tail. Of the three genera Colisa appears the darkest, possessing a total of 131 melanophores in contrast to 57 in Betta, which appears the lightest. In Trichogaster the total number of melanophores is 120. The overall consideration of melanophores of our anabantids shows that Trichogaster trichopterus and Colisa lalia are more closely related than either of them is to Betta splendens. It may be recalled that in the transitional stage of pigmentation Trichogaster trichopterus and Colisa lalia have the same arrangement of melanophores in the tail region. This assumption of relationship is supported very strongly by the species characters exclusive of pigmentation. However, these species all fall into one group by their unique initial site of pigmentation which is the yolk.

Hyphessobrycon serpae in this final stage of pigmentation has an entirely different kind of arrangement of melanophores. Except

for a few melanophores around the eye, the rest cannot be counted because they are highly anastomosed by their processes, giving a net-like appearance. This is found on the yolk, the gut and the ventral edge of the myotome of the tail region. In the tail region the dorso-lateral and mid-lateral lines are represented by very few melanophores. For these reasons the melanophore count is not included in Table X. It would be interesting to discover if such a highly netted appearance of melanophores is peculiar to the species, or to any higher categories.

It is also to be noted that Hyphessobrycon serpae reaches the final stage only after hatching. In this regard it resembles the members of Cyprinodontidae and Anabantidae. It would appear then that the stage of hatching is not of genetic significance.

TABLE X

The number of melanophores at final stage of pigmentation and time of occurrence. This excludes Hyphessobrycon serpae in which the melanophore counts are impossible

Species	Head and nape	Yolk sac and gut	Dorso-lateral line	Mid-lateral line	Ventro-lateral line	Total number of melanophores	Hours after laying	Occurrence before or after hatching
Cyprinidae								
Brachydanio rerio	15	30	35	17	20	117	78	Before
Brachydanio albolineatus	19	31	32	10	19	111	73	Before
Brachydanio nigrofasciatus	14	60	36	16	22	148	49	Before
Tanichthys albonubes	19	24	23	12	27	105	50	Before
Cyprinodontidae								
Oryzias latipes	13	15	28	20	20	96	410	After
Anabantidae								
Betta splendens	14	27	0	0	16	57	92	After
Trichogaster trichopterus	16	30	32	21	21	120	46	After
Colisa lalia	12	45	28	22	24	131	44	After

DISCUSSION

In the present study two kinds of eggs are met with, the pelagic and the demersal. The pelagic eggs, as commonly understood, are those that float or are suspended at various depths, being lighter than water or nearly so. In most pelagic eggs of fishes one or more oil globules are present and these oil globules in normal position remain uppermost. In Trichogaster trichopterus and Colisa lalia (Anabantidae) there is a single large oil globule that remains uppermost in position. These eggs float even without the help of the air bubbles of the nest. Schultz (1955) states that the oil globules help to regulate the specific gravity in pelagic eggs. The eggs of Betta splendens (another anabantid studied) are able to float only when they are adherent to the air bubbles of the nest, otherwise they sink; the male then picks them up and throws them into the nest. This type of egg is difficult to classify because without the support of the air bubbles of the nest it falls to the bottom, behaving like the demersal egg. The air bubbles of the nest of this family seem to serve to buoy up the egg and also to keep the eggs together. Whether the air bubbles of the nest common in the family Anabantidae are necessary for the proper and normal development is not known.

The oil globules found in the highly demersal eggs of Oryzias latipes certainly cannot serve to regulate the specific gravity of

the eggs. Rugh (1948) considers the oil globules of this fish to be nutritive in function, since they disappear when the yolk is absorbed. It might be interesting to determine the function of the oil globules in both the demersal and the pelagic eggs.

According to Roosen-Runge (1938) "an abundant artificial aeration and even circulation of water" is necessary in order to get uniformly healthy eggs in Brachydanio rerio. The present work applies this method, as mentioned earlier, to all the species of Brachydanio; and eggs can be obtained almost every day if these fishes kept in schools are well fed and properly looked after. Whether the air (oxygen) or the current alone or both help to induce the fish to spawn is not known. Mookerjee and Mazumdar (1946c) state emphatically that in Anabas testudineus (Bloch) currents of water are unnecessary for spawning, contrary to the former opinion of the Department of Fisheries. (Bull. 3, 1913, Dept. Fish., Bengal, Behar and Orissa).

Wrinkles (appearing like grooved lines) on the egg-membrane are found in Hyphessobrycon serpae, Betta splendens, Trichogaster trichopterus and Colisa lalia. These are most prominent and regular in arrangement in Hyphessobrycon serpae. In Betta splendens these grooved lines are prominent but without any regular arrangement. In the remaining two fishes the wrinkles are just noticeable. The egg-membrane in the first two fishes is very fragile. It is possible that these wrinkles help to swell the eggs by un-wrinkling when the lines become broader and fainter in later stages of development.

A small dent in the shape of a cone on the egg-membrane with its apex turned towards the interior of the egg, found in Hyphessobrycon serpae, Colisa lalia and trichogaster trichopterus, persisting in a varying degree has been mentioned before. The same structure in Notopterus notopterus is considered a micropyle by Mookerjee and Mazumdar (1946b). It is also mentioned as such in the egg of Carassium auratus L. by Battle (1940). Further verification is needed to ascertain the function of this dent in the fishes studied. As the material is easily available, it should not be a difficult problem to determine the function of this dent.

Though the present work does not aim at inquiry into the origin and formation of color and pigment it will not be out of place to say a few words about these commonly used terms. The terms are vague and non-specific and varying in their usage in different fields of science. Sumner (1937) points out that color is a phenomenon which intrudes into any branch of study in animal biology, physiology, histology, genetics or taxonomy. This worker also states that color and color pattern due to their being the most conspicuous visible characteristics are often used to distinguish one species from another, and that the specific application of the terms "pigment" and "color" awaits further investigation from all angles in order that their usage may be specific and uniform in all studies.

In the field of genetic study on fish color and color pattern, changes during the life history take the form of cyclic emergence and

destruction of melanophores. Goodrich and Anderson (1939) suggest that in the above changes the multiplication of chromatophores is due to the direct gene action and that their destruction is an example of remote gene control of "endocrine regulation". Gordon (1946) and Tavolga (1949) believe that the micromelanophores and macromelanophores found in fishes are controlled by genetical factors; therefore the chromatophores or melanophores may be considered as genetic entities, hence of taxonomic value.

The lack of experimental evidence of the neural crest origin of chromatophores in fishes is probably due to the difficulty in procuring fish eggs of large enough size for experimental purposes, and also due to the easy availability of large amphibian eggs for such studies. According to Norman (1948) the freshwater catfish of Africa, Gymnarchus, has eggs which measure 10 millimeters in diameter. The marine fishes, the species of Arius, Cyclopterus and Annarichas, he further points out, have eggs ranging from two and a half to fifteen millimeters in diameter. If any of these larger eggs could be made available to the workers in this field, the origin of chromatophores in fishes could be properly verified. The present work which deals mainly with the visible melanophores of the developing fish embryos supports also the neural crest origin of melanophores because of their linear arrangement close to the mid-dorsal line and also their movement from this region obliquely downward.

Adult coloration is considered as one of the most important

characteristics by which we distinguish one species from another. However, the exceptions though few are remarkable. As said above it has been pointed out by Norman (1948) that in the Trunkfish (Ostracion sp.) it is extremely rare to find two specimens that are exactly alike in the arrangement of the bands on the body. The coloration in the two sexes of this species is also so unlike that they are often mistaken for different species. Jordan and Evermann (1896) who examined a large number of specimens of a West Indian species, Vaca, (Hypoplectrus unicolor Walbaum) found, contrary to expectation, that there exist extreme variations in coloration in the same areas. This has been described from Havana, from the Florida Keys, and from St. Thomas and St. Croix. The six normal species or "varieties" as he called them were absolutely identical in all respects except in color, the blue, yellow and black being arranged in patterns making it possible to separate them. Schultz (1955) states that different species of fishes show similar coloration in the adult in certain cases. One such example is the case of Danio stoliczkai (Day) and Punctitus ticto (Hamilton=Buchanan). Separation between these two genera is made only on the differences between lateral line, pre-dorsal scales and barbels. It is a recognized fact that the same species of fish under varying conditions of environment exhibits great differences in shade and intensity of coloration. This is particularly true of soles and flounders.

The distribution of pigment in the skin has been explained as due to the incidence of light. Norman (1948) cites the case of a

species African catfish Synodontis, which swims with its belly upward; the coloration of this fish when becoming adult is reversed from the usual, that is, the anatomical dorsal part becomes silvery grey, the anatomical ventral dark brown or black. Fishes living in caves and dark environments are generally almost colorless, but it is not uncommon to find fishes from ocean depths with shades of black, brown or violet. It would be interesting to investigate this exceptional phenomenon. It has been found that cave fish develop pigment if exposed to light. Thus it appears that both light and genetic pattern as well as other possible factors are involved in the phenomenon of animal coloration. From the foregoing examples it may be noted that coloration in the adult is not all-important for species recognition. It becomes necessary to look for other coloration which is more stable and more basic as a significant factor for classification, especially in cases where adult coloration is very similar in closely related species. It was hoped that a better character for species recognition might be found in the embryonic and larval melanophores. Pincher (1947) believes that the pigment pattern of the just hatched young fish is the distinctive feature of the species, whatever the mode of control for pigment formation (light or oxygen). This worker also states that though the young larvae of cod and pollack are very much alike they can be distinguished by their pigment pattern. He further states that the parr marks in the seven-week old salmon fry are more sharply defined than in the trout of the same age, thus readily separating the two. Norman (1948) agrees with Pincher; he

further suggests that these larval features may denote an ancestral character that shows up only in the early developmental stage. Mansueti and Mansueti (1955) further suggest that melanophore embryology should contribute to the identification of especially closely related species. Lagler (1952) recognizes that the specific patterns of melanophores on key areas is also species specific. This fact is pointed out also by Wade (1951) who, where other methods fail in the recognition of the larval species, uses the pattern of melanophores for the separation of some tuna-like larval fishes of Philippine waters, -Auxis sp., Euthynna yaito, Katsuwonus pemalis and Neothunus macropterus.

The study of pigment formation of these fishes, taking into consideration all stages as an overall picture, gives a very strong indication that the embryonic and larval melanophores, basic and fundamental as they are, may provide a means of aiding the taxonomists. Taxonomy has so far relied largely on morphological structures of the adult. The stages taken together show that species (Brachydanio rerio, Brachydanio albolineatus and Brachydanio nigrofasciatus) of a given genus (Brachydanio) are much alike regarding the general distribution of their melanophores, but differ from one another in the detail of pattern. Likewise the genera within a family (both in Cyprinidae and in Anabantidae) agree in having the same initial site of pigmentation. The three genera (Betta, Trichogaster and Colisa) of the family Anabantidae have the yolk sac as the initial site of pigmentation. Anabas

testudineus, another member of this family, also shows the same initial site (Mookerjee and Mazumdar 1946c). Though this observation supports the concept that this feature is indicative as a family character, further information on all members of this family will be required before this can be established as a fact. The two genera (Brachydanio and Tanichthys) of the family Cyprinidae studied have the same initial site of pigmentation as Carassius auratus, another member of this family (loc. cit.). That this character is shared by Oryzias latipes of the family Cyprinodontidae may suggest a relationship of the two families, Cyrpinidae and Cyprinodontidae.

Special attention is called to Hyphessobrycon serpae cited by the majority of the authorities (Table I) as in an order with Cyprinidae. Jordan (1923) alone puts this family (Characidae) into an order (Heterognathi) separate from Cyprinidae (Eventognathi). Our data at least suggest the correctness of Jordan's interpretation.

The present work seems to support the importance of the melanophores of early developmental stages in fishes in indicating taxonomic relationship. The supporting evidence is strongest in what has been called the final stage of pigmentation; in this stage species studied can be recognized by the melanophore counts on key areas, the general distribution and the pattern formation.

SUMMARY

Though the embryonic and larval melanophores were reported in teleost fishes as early as 1909 by Borcea, no progress was made in this field of study until recently. It was the experimental research by Goodrich and Lopashov, and the embryological studies of Hodges and Behre, and Orton that further developed this concept of emphasis on the embryonic melanophores. Recently in the fields of fishery biology Pincher, Norman, Lagler, and Mansueti and Mansueti also recognized the importance of the melanophores as a specific character by which closely related species might be separated.

In Hyphessobrycon serpae, Brachydanio rerio, Brachydanio albolineatus, Brachydanio nigrofasciatus, Tanichthys albonubes, Oryzias latipes, Betta splendens, Trichogaster trichopterus and Colisa lalia no evidence of taxonomic relationship is given by the structure of the egg, blastula, early embryonic shield, pre-pigment stage and hatching stage. Similarities are observed in the species of a genus and in certain genera of a family but these features may be considered adaptive. The time factor is not reliable; there are wide variations.

Pigment patterns are similar in all stages in species of a given genus.

In two genera of the family Cyprinidae initial stages are alike; but later stages differ.

A genus (Oryzias latipes) in another family (Cyprinodontidae)

has the same initial site as the preceding, which suggests possible family relationship of the two genera. In other stages the members of the two families differ significantly.

The three genera of the family Anabantidae are unique not only in the possession of like site of initial pigmentation but also in the later stages. Two genera of the family (Trichogaster trichopterus and Colisa lalia) are more alike in the last two stages than either is to the third genus (Betta splendens). This suggests closer relationship between Trichogaster trichopterus and Colisa lalia than between either and Betta splendens.

Hyphessobrycon serpae of the family Characidae does not resemble any other species at any stage of pigmentation. The majority of the recognized authorities of piscine taxonomy keep the two families (Cyprinidae and Characidae) in a single order; but the family Characidae is placed by Jordan (1923) under a separate order from that of Cyprinidae. Our data at least suggest the correctness of this interpretation.

The overall consideration of the stages of pigmentation in these fishes indicates taxonomic relationship, the indication being strongest in the final stage of pigmentation.

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PLATE I

INITIAL STAGE OF PIGMENTATION

(All figures in this plate and succeeding plates are drawn to the same scale, X 47, except when otherwise mentioned)

Characidae

Fig. 1 *Hyphessobrycon serpae*

Cyprinidae

Fig. 2 *Brachydanio rerio*

Fig. 3 *Brachydanio albolineatus*

Fig. 4 *Brachydanio nigrofasciatus*

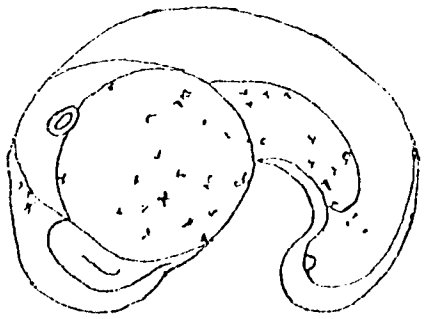
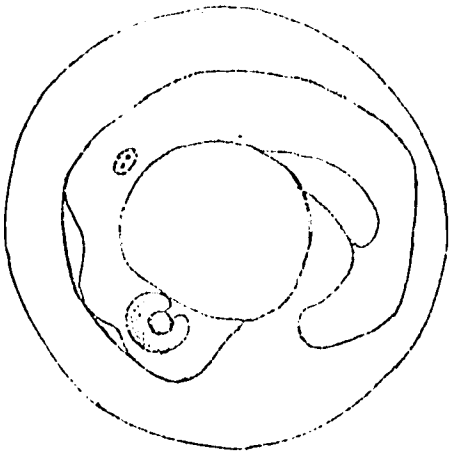


Figure 1



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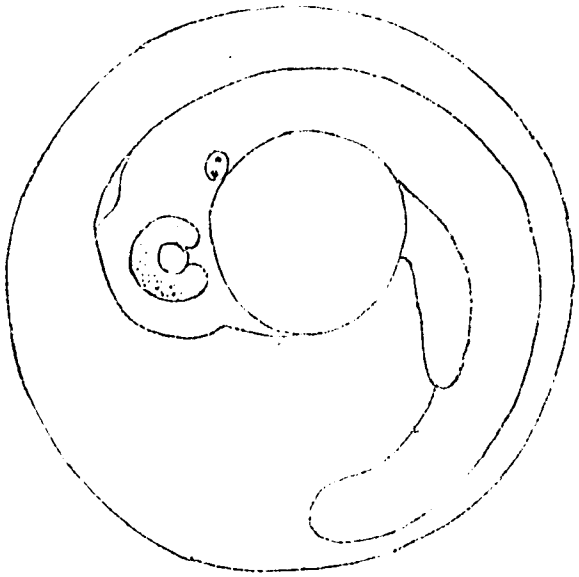


Figure 3

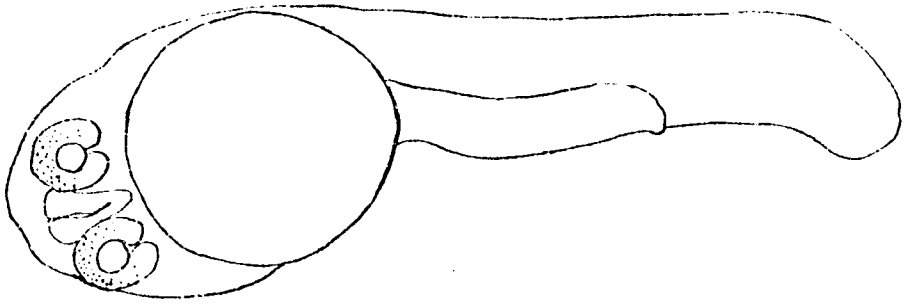


Figure 4

PLATE II

INITIAL STAGE OF FERMENTATION cont'd.

Cyprinidae cont'd.

Fig. 5 *Tanichthys albonubes*

Cyprinodontidae

Fig. 6 *Oryzias latipes*

Anabantidae

Fig. 7 *Betta splendens*

Fig. 8 *Trichogaster trichopterus* (X 74.5)

Fig. 9 *Colisa lalia* (X 74.5)

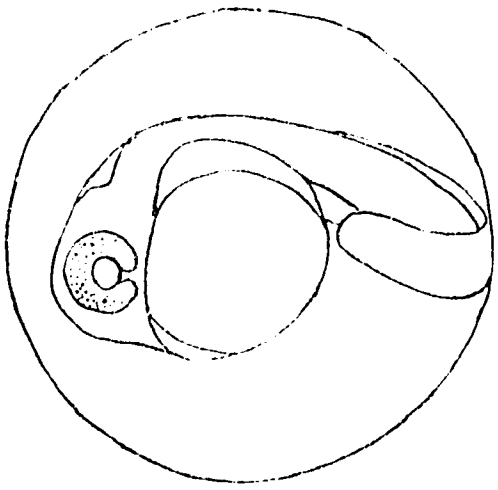


Figure 5

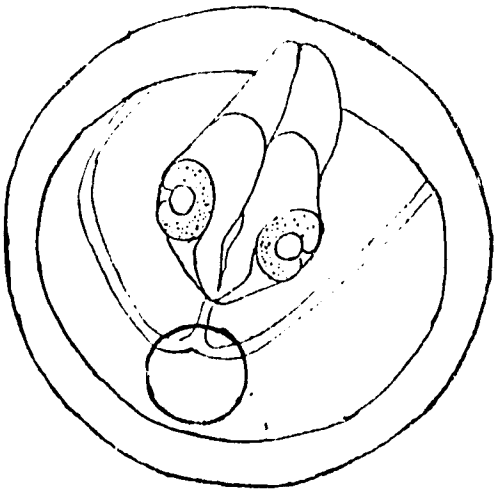


Figure 6

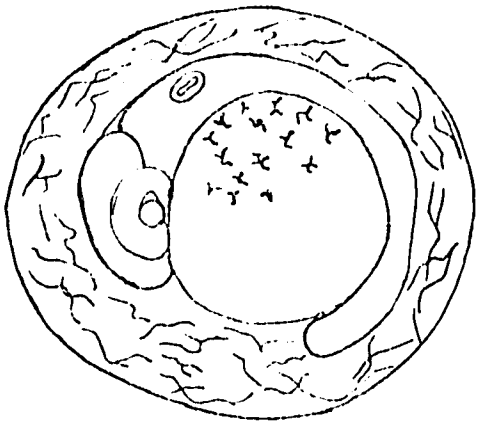


Figure 7

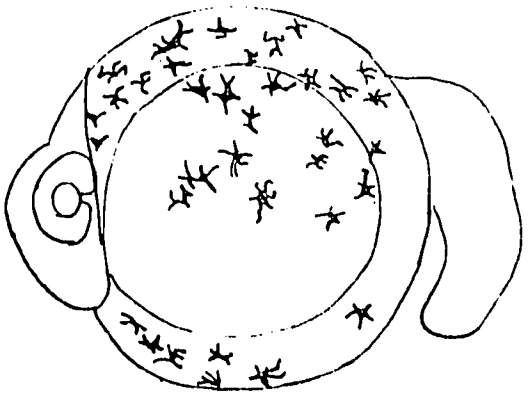


Figure 8

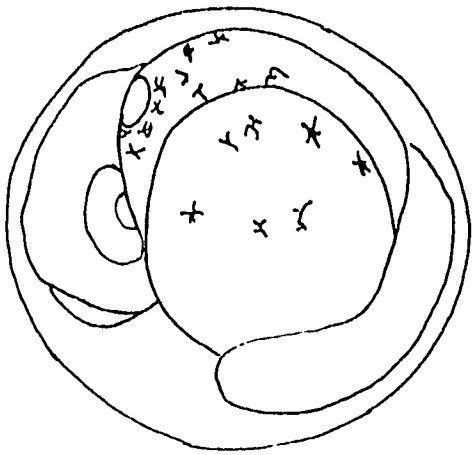


Figure 9

PLATE III

PRIMARY STAGE OF PIGMENTATION

Characidae

Fig. 10 *Hyphessobrycon serpae*

Cyprinidae

Fig. 11 *Brachydanio rerio*

Fig. 12 *Brachydanio albolineatus*

Fig. 13 *Brachydanio nigrofasciatus*

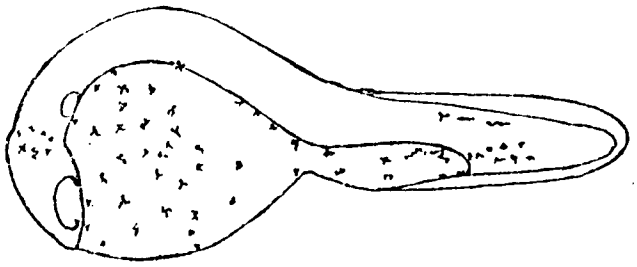


Figure 10

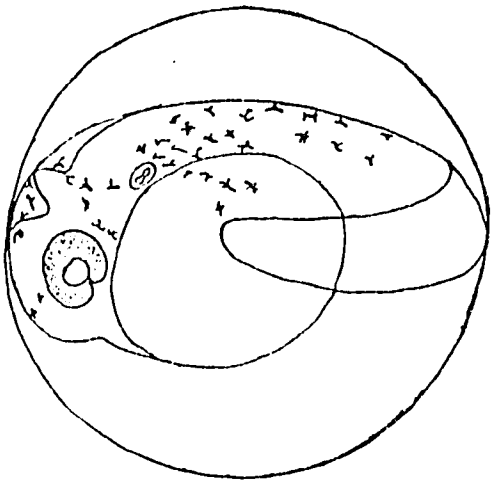


Figure 11

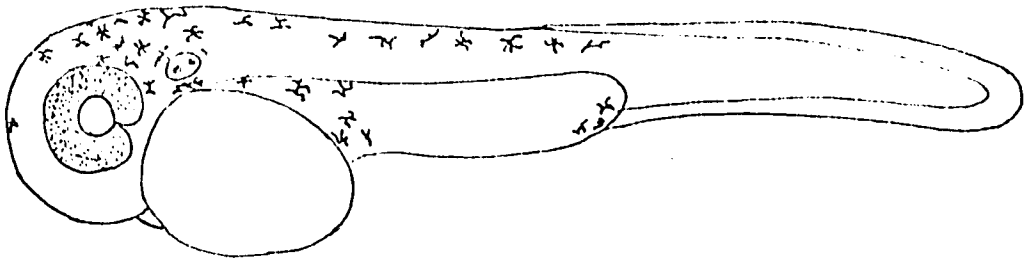


Figure 12

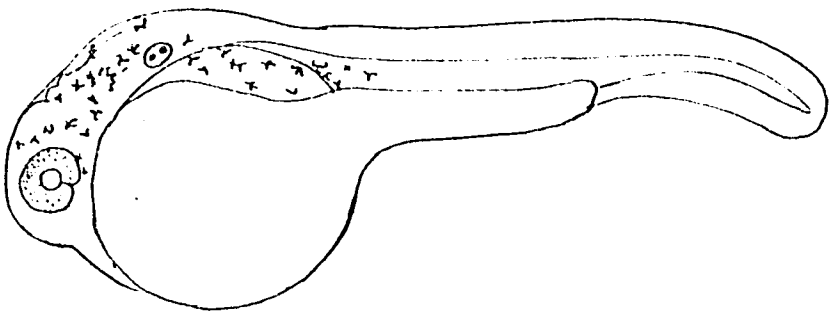


Figure 13

PLATE IV

PRIMARY STAGE OF PIGMENTATION cont'd.

Cyprinidae cont'd.

Fig. 14 Tanichthys albonubes

Cyprinodontidae

Fig. 15 Oryzias latipes

Anabantidae

Fig. 16 Betta splendens

Fig. 17 Trichogaster trichopterus (X 74.5)

Fig. 18 Colisa lalia (X 74.5)

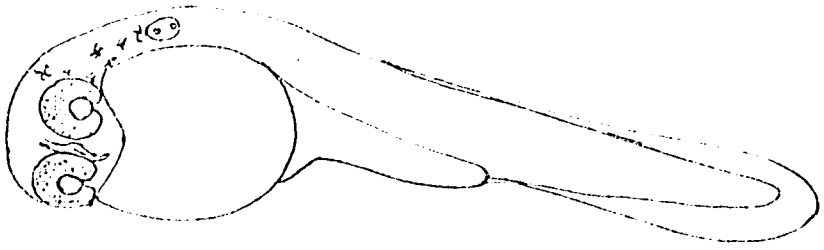


Figure 14

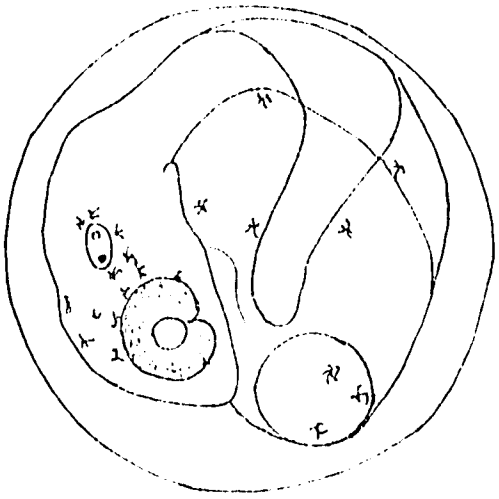
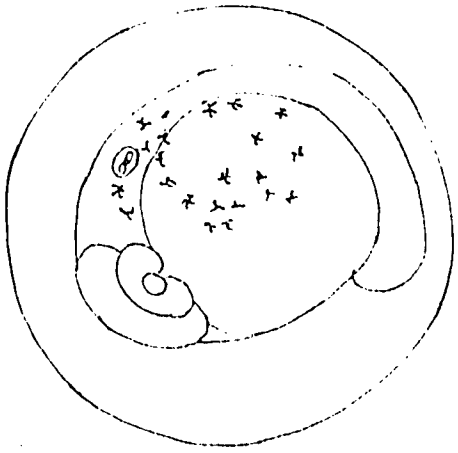


Figure 15



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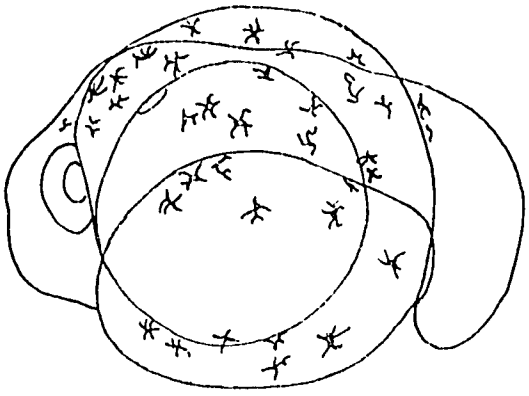

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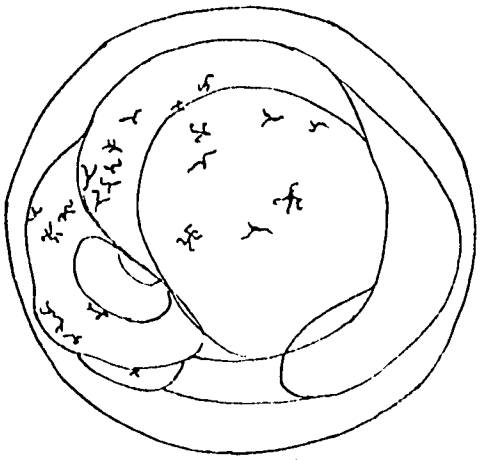


Figure 18

PLATE V

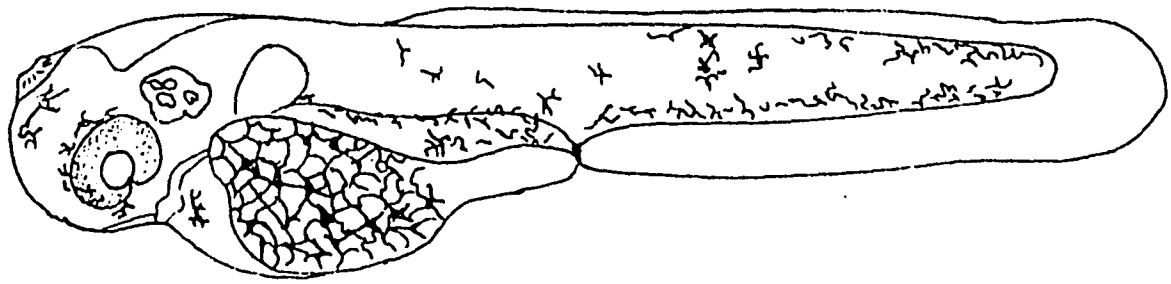
TRANSITIONAL STAGE OF PIGMENTATION

Characidae

Fig. 19 Hyphessobrycon serpae (Note the highly anastomosed melanophores on the yolk sac)

Cyprinidae

Fig. 20 Brachydanio rerio



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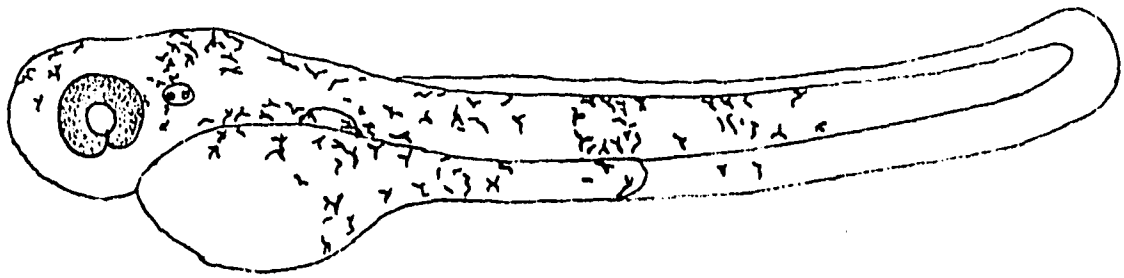


Figure 20

PLATE VI

TRANSITIONAL STAGE OF PIGMENTATION cont'd.

Cyprinidae cont'd.

Fig. 21 Brachydanio albolineatus

Fig. 22 Brachydanio nigrofasciatus

Fig. 23 Tanichthys albonubes

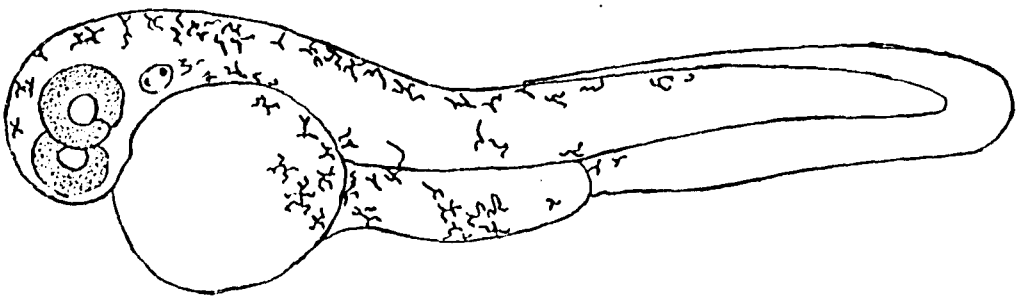
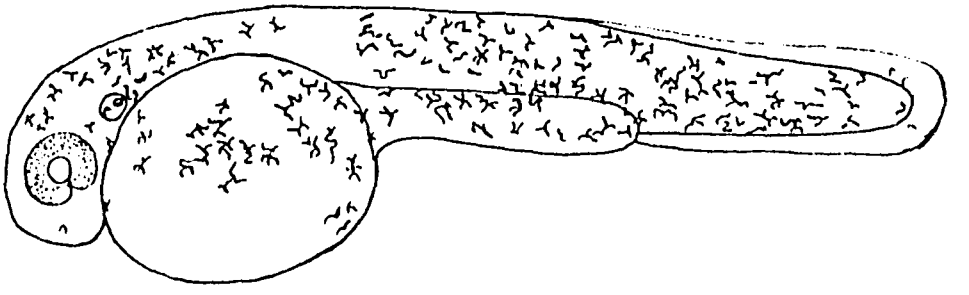


Figure 21



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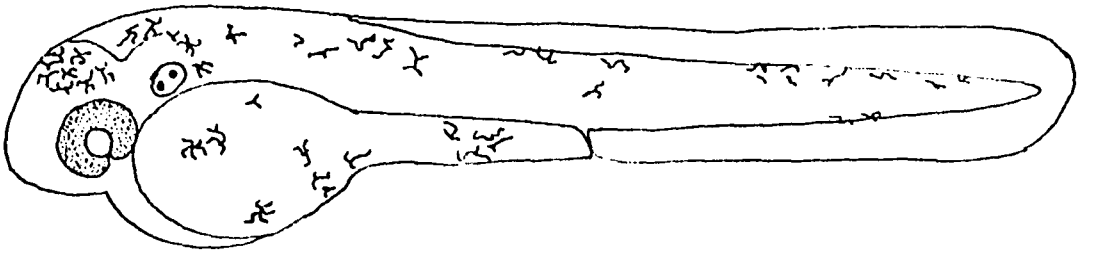


Figure 23

PLATE VII

TRANSITIONAL STAGE OF PIGMENTATION cont'd.

Cyprinodontidae

Fig. 24 Oryzias latipes

Anabantidae (Note increasing differences in the pigment pattern of this family)

Fig. 25 Betta splendens

Fig. 26 Trichogaster trichopterus (X 74.5)

Fig. 27 Colisa lalia (X 74.5)

Figure 24

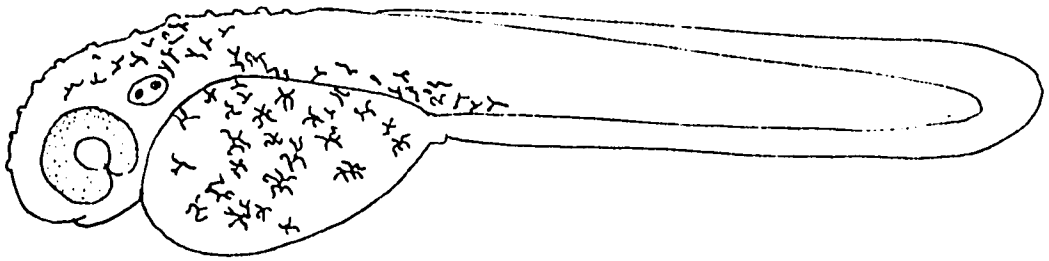
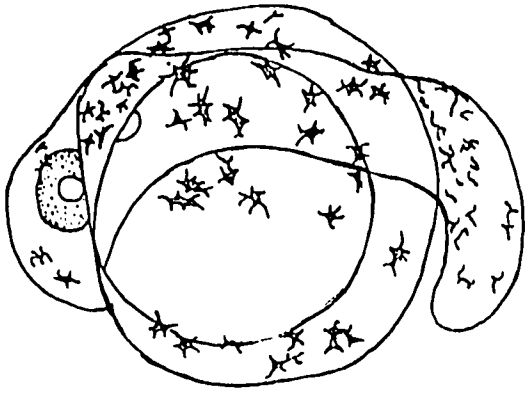


Figure 25



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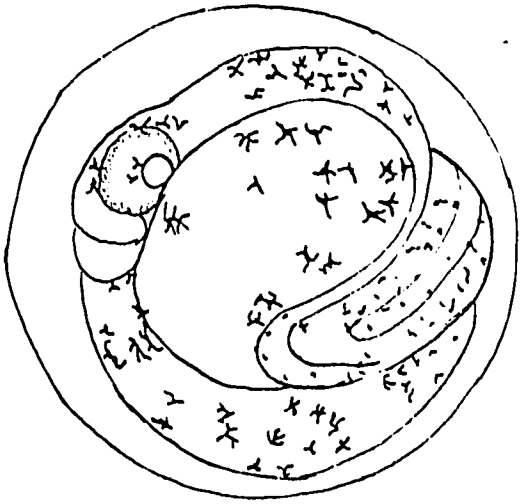


Figure 27

PLATE VIII

FINAL STAGE OF PIGMENTATION

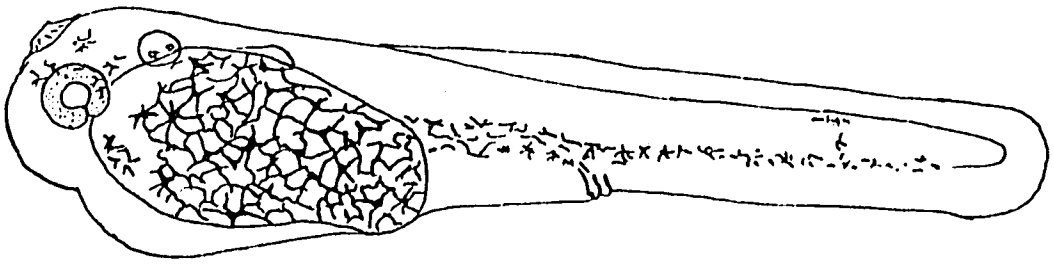
Characidae

Fig. 28 *Hyphessobrycon serpae*

Cyprinidae

Fig. 29 *Brachydanio rerio*

Fig. 30 *Brachydanio albolineatus*



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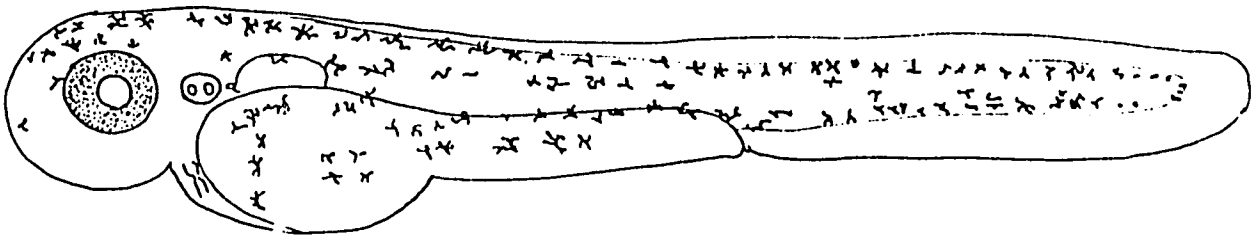

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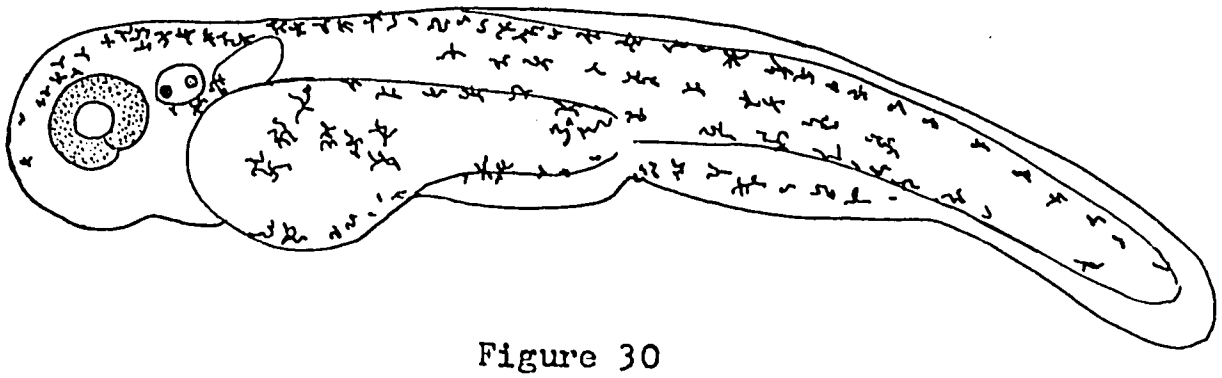


Figure 30

PLATE IX

FINAL STAGE OF PIGMENTATION cont'd.

Cyprinidae cont'd.

Fig. 31 Brachydanio nigrofasciatus

Fig. 32 Tanichthys albonubes

PLATE IX

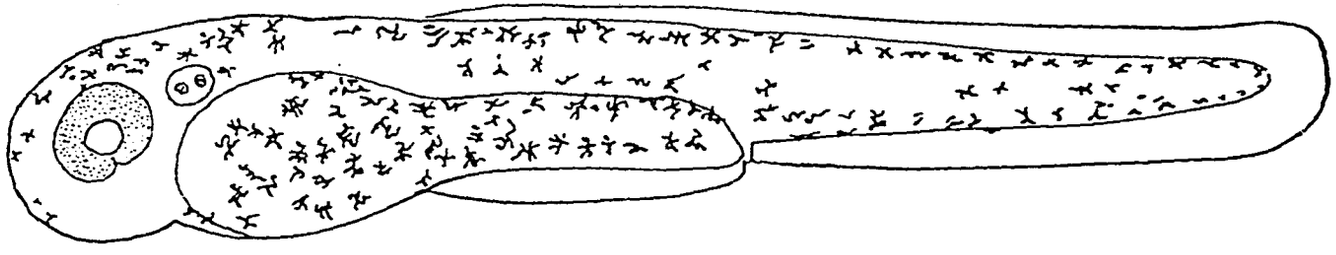


Figure 31

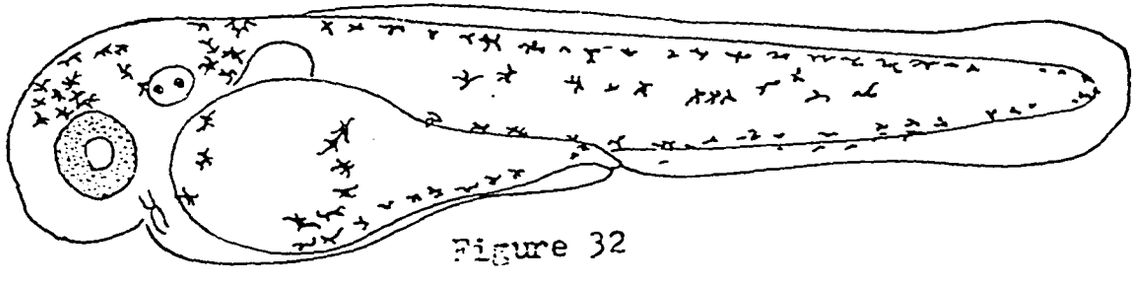


Figure 32

PLATE X

FINAL STAGE OF PIGMENTATION cont'd.

Cyprinodontidae

Fig. 33 Oryzias latipes

Figure 33

PLATE X

PLATE XI

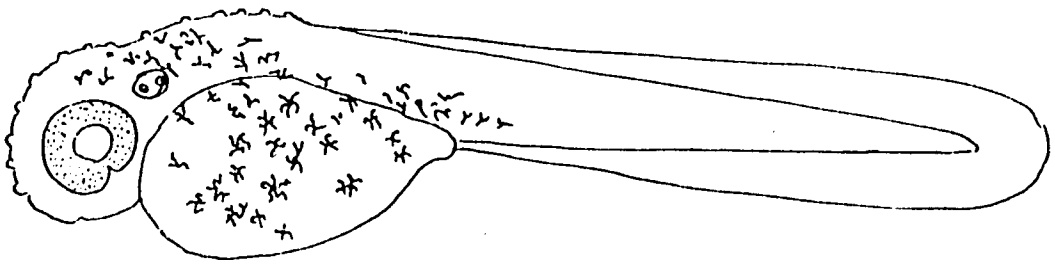
FINAL STAGE OF PIGMENTATION cont'd.

Anabantidae

Fig. 34 Betta splendens

Fig. 35 Trichogaster trichopterus

Fig. 36 Colisa lalia



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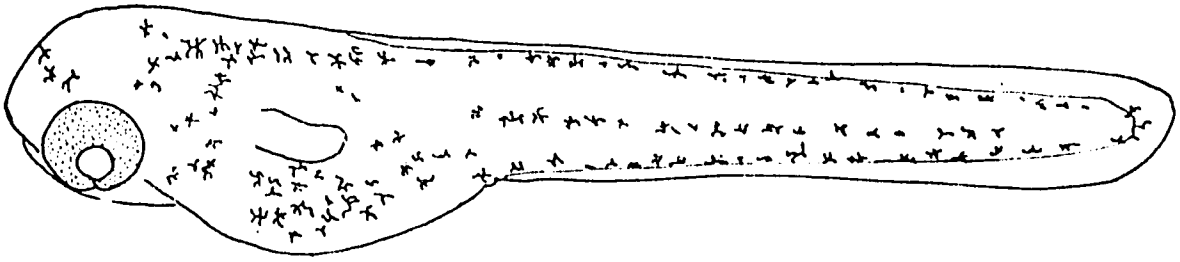

Figure 35

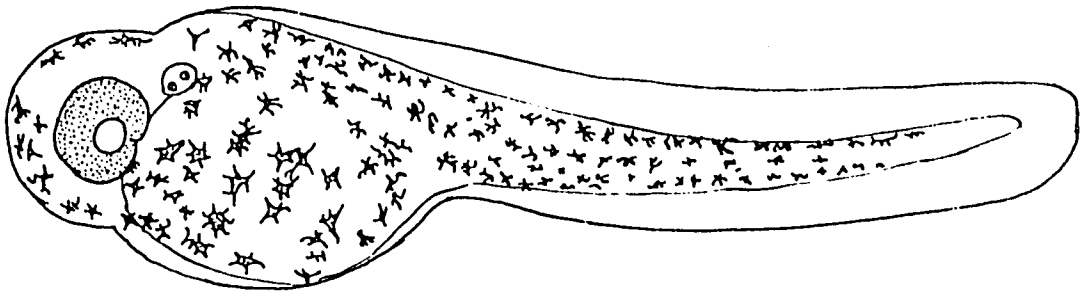

Figure 36

AUTOBIOGRAPHY

Saw Tha Myint was born on March 10, 1910, in Hanthawaddy District, Burma. He was graduated from Judson Boys' High School, Moulmein in March, 1930. He entered the University of Rangoon in the same year and passed the Intermediate of Science Examination in March, 1932. He continued college work and completed the courses leading to the Bachelor of Science degree in 1934; since then he went to teach in Cushing High School, Baptist English High School and Judson Boys' High School until Burma became a battle field of the Second World War in 1941. In 1938 he passed his Higher Grade Teachership Examination held by the Directorate of Public Instructions, Burma. In August 1947 he passed the Bachelor of Science degree examination from the University of Rangoon and was taken onto the teaching staff of the Department of Biology. In March, 1952, he then obtained the Master of Science degree with Zoology as his major subject. He was given a study leave in August 1954 by the University of Rangoon to take up further training in the United States of America. He entered the Graduate School of Louisiana State University, in September 1954.

At present he holds a term of deputation given by the University of Rangoon, and is a candidate for the degree of Doctor of Philosophy in the field of Zoology.

EXAMINATION AND THESIS REPORT

Candidate: Saw Tha Myint

Major Field: Zoology

Title of Thesis: Embryological Studies of Certain Teleost Fishes with Special Reference to the Possible Significance of Melanophores in Piscine Taxonomy

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 20, 1956